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MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

II. Bacteria Concerned in the Cycle of Nitrogen in the Sea[1]

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Introductory

Among the various elements which are essential for the growth of plant and animal life in the sea and which are present in minimum concentrations, nitrogen occupies a prominent place. Neither the plant nor the animal forms are able to use the gaseous nitrogen of the atmosphere, a function now known to be limited chiefly to certain specific groups of bacteria, and depend for their growth upon the combined nitrogen present in the sea; but even this form of nitrogen must be in a soluble, mineralized state before it can be assimilated by the plants. The plants transform the soluble forms of nitrogen into complex organic forms; upon the death of the plants, as well as of the animals which feed upon them, the nitrogen is brought again into circulation, through the action of a number of bacteria, in the form of ammonia. However, before this nitrogen is again assimilated by the marine plants, the ammonia is usually oxidized first to nitrite and then to nitrate by the action of certain other bacteria. The nitrate, if not consumed by the plants especially in the surface layers of the sea, may be reduced, under certain conditions, by the action of still other bacteria to nitrite, to ammonia, and to gaseous forms of nitrogen. Some bacteria may compete in the sea with higher plants for the available inorganic nitrogen, in the presence of certain carbohydrates, and thereby transform this nitrogen into organic compounds.

The bacteria are thus concerned in the sea with at least five distinct

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processes which are based upon the circulation of nitrogen, and which affect in numerous ways, directly and indirectly, the growth of plant and animal life in the sea, namely (1) liberation of nitrogen as ammonia, (2) oxidation of ammonia to nitrite and nitrate, (3) reduction of nitrate to nitrite and atmospheric nitrogen, (4) fixation of nitrogen, and (5) assimilation of inorganic forms of nitrogen. The first and last of these processes have been discussed at some length in a previous publication (48). The investigations reported in this paper are concerned with the other three processes of nitrogen transformation.

Occurrence of Nitrifying Bacteria in the Sea

Historical

The formation of nitrate in the sea, as a result of oxidation of ammonia, is usually considered as the final step in the transformation of nitrogen, before it is made again available for assimilation by green plants; this does not exclude, of course, the probability that ammonia itself, the final nitrogenous product of decomposition of organic matter by bacteria, and nitrite, the first product of oxidation of ammonia, can also be utilized by marine algæ and by the chlorophyl-containing members of the plankton, as sources of nitrogen. Among the various marine problems, for which bacteria are believed to be responsible, none has aroused more discussion and greater interest than the process of nitrate formation in the sea, with the possible exception of nitrate-reduction by bacteria. This interest is due to the importance of nitrate formation in the metabolism of the sea and to the difficulty of studying the bacterial agents responsible for this process. An exact analogy is found in the study of the agents of nitrification on land: even forty years after the respective organisms have been isolated and cultivated by Winogradsky, papers still continue to appear which not only question the rôle of these organisms in the process but frequently doubt their very existence.

Boussingault looked upon the ocean, in 1860, as an immense reservoir of nitrogen in a combined form. Schlösing (43) demonstrated, in 1875, that while land waters are richer in nitrate, sea waters are richer in ammonia. It was recognized, however, that both ammonia and nitrate result from the decomposition of nitrogenous organic matter in the sea. Natterer (36) measured the NH_3, NO_2, and NO_3 content of sea water and found that the first was most abundant, while the last two were present in mere traces or were entirely absent; he believed that nitric acid produced by electric discharges sooner or later reaches the sea, enters there into organic combination, and is finally transformed to ammonia; this diffuses then into the atmosphere, and

contributes again to the growth of plants on land; these results were not confirmed, however, by subsequent investigators. Brandt (9, 10) stated emphatically that the cycle of nitrogen in the sea is essentially not very different from that on land.

The first suggestion concerning the existence in the sea (Gulf of Naples) of bacteria responsible for the process of nitrification was made in 1898 by Vernon (45). Baur and Brandt (2) are believed to have demonstrated in 1900 the presence of nitrifying bacteria in the sea: two out of three mud samples inoculated into a solution containing ammonium salts gave active nitrification; however, sea water itself seemed to be free from the organisms concerned in this process. These results could not be confirmed by Gran (19) and Nathanson (37), who were unable to demonstrate these organisms either in the Norwegian fjords or in the Gulf of Naples. Gran found nitrifying bacteria only close to the shore. This led Nathanson to conclude that when bacteria are found in the sea not far from land, it is due to their introduction from the land soils by streams and land drainage. Gran and Nathanson adhered, therefore, to the earlier hypothesis of Schlösing that nitrates are not formed in the open sea, but are brought there from the outside, either from the atmosphere or from land.

Brandt argued that, if the nitrate comes into the sea either from the atmosphere or from land by means of streams and rivers, one would expect to find nitrate more abundant in the surface layers of the sea rather than in the lower depths, which is contrary to actual facts. The abundance of nitrate in the deeper layers of water led Brandt to conclude that nitrification takes place chiefly in the sea bottom or close to it. It was recognized, however, that close to the mouth of the rivers the relative concentration of nitrate was greater than in the open sea, but this was also found to hold true for other forms of nitrogen, namely ammonia and protein.

Thomsen (44) demonstrated the presence of nitrite-forming bacteria in considerable abundance in the sea bottom, although they were absent in the sea water and on algal material; the nitrate-forming organisms were also present in the bottom material, but only close to shore. The organisms responsible for the two processes, namely *Nitrosomonas* and *Nitrobacter,* were isolated from the sea and were found to be morphologically the same as the corresponding forms isolated by Winogradsky from land soil. The marine nitrite bacteria were considered as adaptation forms, their optimum temperature being similar to that of the bacteria from land. Thomsen found nitrifying bacteria not only in the mud from the Gulf of Naples, but also from the Kiel Bay and the North Sea. He believed that the negative results of

Nathanson were due to the nature of the medium which he had used for demonstrating the presence of these organisms, and to the fact that the cultures were not incubated sufficiently long and that the temperature was unfavorable. The occurrence of these bacteria on the bottom of the sea and not in the free water itself was explained by the fact that the water contains only traces of ammonia, while the continuous decomposition of plant and animal residues in or on the bottom supplies the necessary substrate for their action.

Issatchenko (24, 25) reported in 1908 that he found nitrifying bacteria in the water of the northern Arctic Sea; these organisms were present in the bottom material of the Catherine Coast (Murmansk) and of the North Ice Sea, as well as of the high seas; they were absent, however, in the surface water. Only nitrite-forming bacteria were found in the sea bottom, but not the nitrate-formers. In a later contribution (26), Issatchenko has shown that the nature of the sea bottom material is of importance in determining the abundance of nitrifying bacteria; these organisms were more abundant and could be more readily demonstrated in sandy bottoms and in shell-rich bottoms than in clay bottoms.

Liebert (30) attempted, in 1915, to isolate nitrifying bacteria from the water of high seas and from the ocean bottom; his results were entirely negative. He concluded that sea waters at a distance from land contain no bacteria capable of oxidizing ammonia and nitrate, due to the low content of these nitrogenous compounds in the sea. Marine mud from the North Sea also gave negative results, except in close proximity to shore. However, both nitrite and nitrate formation took place in the Zuyder Sea. Liebert believed that the FeS present so abundantly in the bottom of the ocean may play an important rôle in the oxidation of the ammonia in marine bottoms. Berkeley (14), in 1919, tested sea water for the presence of nitrifying bacteria with negative results, even after three months incubation of the cultures. As a result of a series of investigations, Lipman (31) concluded that although nitrifying bacteria are absent in the water of the open sea, they are present in the sea bottom, such as calcareous sand. Similar results were reported by Harvey (21–23).

On the basis of these results, Brandt (11) concluded in 1926, with much justification, that the results so far obtained are sufficient to establish definitely the fact that bacteria capable of oxidizing ammonium salts are completely lacking in surface waters, but are present in marine bottoms.

The possibility of photo-chemical oxidation in the sea of ammonia to nitrite and even to nitrate has recently been suggested (51). It has

been known (39) that solutions of ammonia and ammonium salts exposed to sunlight, in the presence of small quantities of a photo-sensitizer, will give rise to nitrite, especially in alkaline solutions. TiO_2, ZnO, CdO, and others act as photo-sensitizers. The photochemical formation of nitrite and nitrate can, at best, however, explain only partly the origin of nitrates in the sea, since it must take place in the surface layers, with the formation of ammonia preceding this process. The fact that the nitrate is largely found in the lower layers of water would tend to emphasize the probable limitation of this process in the formation of nitrate in the sea.

Experimental

In an attempt to study the occurrence and activities of nitrifying bacteria in the sea, it was deemed essential to establish at first the conditions favorable for the growth of these organisms. One deals here with bacteria highly selective in their metabolism, very sensitive to environmental conditions, and specific in their food requirements, as was amply shown for the corresponding organisms universally active and abundant in land soils. Whether or not the bacteria responsible for nitrite and nitrate formation in the sea are as highly sensitive to conditions as the land bacteria, the first prerequisite in such investigations was to select a medium favorable for their development under artificial laboratory conditions. Several preliminary experiments were, therefore, carried out in order to test sea water as a medium and the most optimum conditions for the growth of these organisms.

At first, fresh sea water, to which 0.05 per cent K_2HPO_4, some $CaCO_3$, and varying amounts of ammonium salts had been added, was used. These experiments have shown that neither nitrate nor nitrite was formed in such a medium. However, when fresh sand or mud was introduced into the flasks, active nitrite formation took place. The results of a typical experiment are reported in Table I. The medium used in this experiment consisted of,

Fresh sea water	1000 cc.
K_2HPO_4	1 gram
$(NH_4)_2SO_4$	1 gram
$CaCO_3$	5–10 grams

This medium was placed in a series of flasks; these were plugged with cotton and the medium left unsterilized. Some of the flasks received 30-gram portions of fresh bottom mud taken off the shore of Gay Head, while others did not receive any mud. At frequent intervals nitrite tests were made by removing 1-cc. portions of the culture, by the use of sterile pipettes. The test was made with a mixture of sulphanilic

acid and diethyl β-naphthylamine solutions. One cc. of culture was diluted with 5 cc. of distilled water and 1-cc. portions of each of the two reagents added. The color was read after 5 or 10 minutes.

The results brought out in Table I clearly demonstrate the fact that sea water either does not contain any bacteria capable of oxidizing ammonium salts to nitrite or is not a favorable medium for the development of these bacteria. In the culture to which marine mud has been added active nitrite formation took place, even after 20 days incubation; the amount of nitrite formed increased rapidly on further incubation. This can be due either to the presence of nitrifying bacteria in the mud or to the fact that the mud made the sea water medium more favorable for the development of these organisms.

TABLE I

Nitrite formation in sea water medium and in sea water medium to which fresh marine mud has been added.

Tr = trace of nitrite; 0 indicates negative test; + = positive nitrite test; +++ = extensive nitrite formation; ++++ = maximum nitrite reaction; − indicates culture discarded.

Treatment of culture	Days of incubation of cultures						
	6	15	20	24	28	33	38
Water alone	0	0	0	0	tr	tr	tr
Water alone	0	0	0	0	tr	−	−
Water and mud	0	0	+	+++	++++	++++	++++
Water and mud	0	0	+	+++	++++	−	−

Another important point to be noted from this experiment is that only the tests for nitrite were positive, while the tests for nitrate were all negative, even in the mud cultures. Attention has been called, in the review of the literature, to the comparative rarity in the sea of the nitrate-forming bacteria, as compared with the nitrite-formers. This would seem to be an anomaly, since there is very little nitrite present in the sea as compared with nitrate. If one remembers, however, the fact that the nitrate bacteria are highly sensitive to traces of free ammonia, which would be produced from the ammonium salt in an alkaline medium, one would expect to find these organisms, if they are present at all, only after all the ammonia has been oxidized to nitrite. The tests were continued for a longer period, but no trace of nitrate could be detected within 57 days of incubation; however, after 60 to 62 days, the mud containing cultures gave a definite test for nitrate, and after 77 to 84 days, the test became strongly positive.

An experiment was then started to determine the influence of the

initial concentration of ammonium salt added to the original medium upon the development of nitrite and nitrate-forming bacteria, since some of the previous investigators (31, 33) considered this to be an important factor. This experiment was carried out as follows: Twelve 250-cc. Erlenmeyer flasks received 40-gram portions of washed sea sand and 2 grams $CaCO_3$; the flasks were plugged with cotton and sterilized for 1 hour under pressure. Sixty-cc. portions of sea water containing 0.1 per cent K_2HPO_4, previously heated for 1 hour at 70° C., were then added to the flasks, as well as varying amounts of an ammonium sulfate solution, also heated previously at 70° C. Five of the flasks were inoculated with fresh surface water taken from Great Harbor,

TABLE II

Effect of varying concentrations of ammonium sulfate upon nitrite formation in sea water-sand medium.

0 indicates negative test; + = positive nitrite test; ++ and +++ = extensive nitrite formation; ++++ = maximum nitrite formation.

Concentration of $(NH_4)_2SO_4$ in 60 cc. of medium	Sea water inoculum					Mud inoculum					Enriched culture inoculum			
	Days of incubation					Days of incubation					Days of incubation			
	3	7	12	17	20	3	7	12	17	20	3	7	12	17
mgm.														
0	0	0	0	0	0	0	0	+	++++	++++				
10	0	0	0	0	0	0	0	++	++++	++++				
25	0	0	0	+	+++	0	0	0	+	++	0	0	+	++++
50	0	0	0	+	++	0	0	+	+++	++++	0	0	+	++++
100	0	0	0	0	0	0	0	0	++	++++				

near the Oceanographic Institution wharf, five flasks were inoculated with sea bottom mud from off the shore of Gay Head and 2 flasks with 4 drops of an enriched culture of nitrite-forming bacteria grown in a sand-sea water medium.

The concentration of the ammonium salt and the nature of the inoculum were found to have an important effect upon the rapidity of nitrite formation, as shown in Table II. The use of sea water as an inoculum gave no nitrite formation with either the highest or the lowest concentrations of the ammonium salt; however, a positive reaction was obtained in the cultures containing 25 and 50 mgm. of the salt, after 17 days incubation. The cultures inoculated with mud gave a positive nitrite test in some cultures in 12 days and in all cultures in 17 days. The mud cultures behaved in a manner similar to the enriched culture of the nitrite-forming organism.

Some of the cultures in the above experiment were incubated for a longer period than that reported in Table II. The culture containing 10 milligrams of ammonium sulfate and inoculated with mud gave very abundant nitrite formation up to 34 days; after 42 days, however, the nitrite disappeared. It had been completely and rapidly converted to nitrate.

In the previous experiments, both the water and the marine bottom material were obtained in the proximity of land. The following ex-

TABLE III

Presence of nitrite-forming bacteria in the sea water of the Gulf of Maine (Stations 1329, 1330, 1331, and 1332) and Georges Bank (Stations 1333 and 1334) at different depths.

Tr = trace; 0 indicates negative test; + indicates positive reaction; ++ = extensive nitrite formation; +? indicates doubtful reaction. For map showing location of stations, see Rakestraw, 1933, *Biol. Bull.*, **64**: 150.

Station No.	Depth of water	Days of incubation			
		10	15	20	23
	meters				
1329	Surface water	0	+?	0	0
1329	Above bottom	0	0	0	0
1330	Above bottom	0	0	0	0
1331	Surface water	0	0	0	0
1331	30	tr	tr	tr	+
1331	50	tr	+	+	+
1331	100	tr	0	0	+
1331	215	tr	+	+	+
1331	Above bottom	0	0	0	0
1332	Surface water	0	+	+	++
1332	50	0	+	+	+
1332	100	0	+	+	+
1333	Surface water	0	tr	tr	tr
1334	Surface water	0	tr	0	tr
1334	Deep water	0	tr	tr	tr

periments deal with the occurrence of nitrifying bacteria in the Gulf of Maine and on George's Bank, at a considerable distance from shore. Material for this experiment was obtained on a cruise of the "Atlantis," which took place during August 1–5, 1932, and which has been described in detail elsewhere (50). For this purpose a series of flasks were prepared containing the following materials:

Sand	15	grams
$CaCO_3$	1	gram
K_2HPO_4	0.005	gram
$(NH_4)_2SO_4$	0.01	gram

The flasks were plugged with cotton and sterilized, at 15 lbs. pressure,

for 15 minutes. A standard solution of ammonium sulfate was sterilized separately and added to the sterile flasks. At the various stations visited about 50–60-cc. portions of fresh sea water, brought up from the different depths, by means of sterile glass containers, were added to the flasks immediately after the samples were obtained. To determine the occurrence of nitrifying bacteria in the marine bottom of the open sea, a medium similar to the above and containing 75-cc. portions of sea water with only 5 milligrams of $(NH_4)_2SO_4$ per flask was prepared. This medium was sterilized for 1 hour in flowing steam (100° C.). The flasks were inoculated, after returning from the cruise, with small quantities (about 1 gram) of fresh mud, obtained in the Gulf of Maine under sterile conditions; this was done by removing carefully the inner part of a core of mud.

TABLE IV

Occurrence of nitrite-forming bacteria in the marine bottom of the Gulf of Maine (Stations 1329 and 1330) and Georges Bank (Station 1336).

Tr = trace of nitrite; + = positive nitrite test; ++ and +++ = extensive nitrite formation. For map showing location of stations see Rakestraw, 1933, *Biol. Bull.*, 64: 150.

Station No.	Depth of mud	Days of incubation			
		2	6	11	15
	cm.				
1329	0–30	+	++	+++	+++
1329	30–60	0	++	++	+++
1329	60–90	0	tr	tr	tr
1330	0–30	+	+++	++	+++
1330	30–60	0	tr	tr	tr
1330	60–90	tr	tr	tr	tr
1336	Surface layer of sand bottom	0	+	+	++
Control	0	0	0	0	

The results of these experiments on the presence of nitrite-forming bacteria in the sea water and in the marine bottom of high seas, are reported in Tables III and IV. These results show that the free water in the high seas is either entirely free from nitrifying bacteria or contains only very few cells of these organisms. In the case of the marine bottoms, however, positive nitrite formation was obtained in the case of the mud bottoms (Stations 1329 and 1330). Even within two days incubation these bacteria seem to be present in the mud to quite considerable depths. The sand bottom also gave active nitrite formation, somewhat more slowly than the mud bottoms.

In addition to the cultures containing ammonium salts, other cul-

tures containing nitrites were prepared, but all attempts to obtain nitrate-forming bacteria in these media failed. However, when old cultures of nitrite-forming bacteria, in which nitrate-formation began to take place, were transferred to fresh media containing 5 mgm. KNO_2 in 30 cc. of medium, active nitrate formation took place within 7–8 days.

The following medium was finally adopted for the purpose of demonstrating the presence of nitrifying bacteria, using large test tubes instead of flasks, due to the ease of handling. The tubes contained 30 cc. sea water, 10 gm. washed sand, 1 gm. $CaCO_3$, 5 mgm. $(NH_4)_2SO_4$, and 5 mgm. K_2HPO_4. The medium was sterilized in flowing steam; the ammonium salt was sterilized separately, then added in aliquot portions, using sterile pipettes.

The nitrite-forming organism could be readily cultivated on the above medium; on repeated transfer, active cultures were obtained. By inoculating a silica-gel medium (thoroughly dialyzed in tap water and soaked in sterile sea water), to which an ammonium salt and calcium carbonate had been added, with an active liquid culture, abundant formation of nitrite on the plate took place within 14 days. This should facilitate greatly the isolation of the organism in pure culture, although for the purpose of the above experiments this was not considered essential.

The results presented here and other data of a similar nature are quite sufficient to demonstrate definitely that free sea water, especially at the surface of the sea, has either no nitrifying bacteria at all or only very few of these organisms. On the other hand, the sea bottom, mud or sand, has an active population of nitrifying organisms. The formation and accumulation of nitrate in the sea is probably due largely to the activities of these organisms. The processes of nitrite and nitrate formation take place in the sea bottom; the nitrate then diffuses into the water. The fact that it is much easier to demonstrate in culture the formation of nitrite than that of nitrate is due largely to the specificity of the organisms and conditions of cultivation.

Reduction of Nitrates in the Sea by Bacteria

Historical

Nitrate is consumed in the sea not only by the green plants but also by bacteria, which either transform it into bacterial cell substance or reduce it to nitrite, ammonia, or atmospheric nitrogen. The activities of the last group of bacteria have aroused the interest of oceanographers. The increasing concentration of nitrate with the depth of the water in the open sea, the high nitrate content in temperate waters and

its comparative poverty in tropical waters, the fact that nitrate is the final product of the transformation of nitrogen by bacteria, and further the fact that the phytoplankton and marine algæ seem to use this form of nitrogen in preference to any other, have all served to center the attention of marine investigators upon the transformation of nitrate in the sea. An attempt has actually been made to correlate not only the relative abundance of nitrate in the sea but also the abundance of plant and animal life with the activities of the nitrate-destroying bacteria.

If one is to admit the assumption that bacteria control largely the cycle of nitrate in the sea, especially its disappearance under certain conditions and not under others, one would have difficulty in explaining the common observation that nitrate and phosphate occur in the sea in a parallel manner. If one were to conclude that the nitrate balance in the sea is controlled by the activities of nitrate-forming and nitrate-reducing bacteria, one must also postulate that the formation and disappearance of phosphate in the sea are also controlled by the activities of comparable groups of bacteria. This may be true for the liberation of nitrate and phosphate, since nitrate formation is a resultant of a chain of processes brought about by a series of bacteria, while phosphate is liberated directly, in the decomposition process of plant and animal residues in the sea. On the other hand, although nitrate-reduction is known to take place under favorable environmental conditions, which include the presence of nitrate, an available source of energy, and the necessary bacteria, the reduction of phosphate in the sea, aside from its direct consumption by the phytoplankton, is still a matter of conjecture.

It is further essential to keep in mind the fact that the nitrate-reducing bacteria do not comprise a single group of organisms but several groups with distinct physiological properties, namely, (*a*) bacteria which reduce nitrate to nitrite only, (*b*) bacteria which reduce nitrate and nitrite to ammonia, and (*c*) bacteria which reduce nitrate and nitrite to atmospheric nitrogen and to gaseous oxides of nitrogen. Only the last group should be considered as true denitrifying bacteria; their activities may be of significance in the disappearance of nitrate in the sea. The products formed by the first two groups of nitrate-reducing bacteria can either be assimilated directly by the marine algæ and the phytoplankton or they can be oxidized back to nitrate by specific bacteria.

According to Audoynaud (1), both ammonium salts and nitrates are assimilated by the numerous small organisms inhabiting the sea, their bodies later sinking to the bottom and forming layers of various degrees of thickness. He, as well as Schlösing (43), had only a very

vague idea concerning the return of the elements, especially of the nitrogen, to the atmosphere or to the sea, as a result of the decomposition of the organic residues.

The presence in the sea of bacteria capable of reducing nitrate, largely to nitrite, was demonstrated by Beijerinck (5) in 1890, by Fischer (15) in 1894, for phosphorescent bacteria, by Russell (42) in 1893, for the sea in the vicinity of Woods Hole, and by Vernon (45) in 1898, for the waters in the Gulf of Naples.

A decided stimulus to further investigations of nitrate-reducing bacteria was given by Brandt (9), in 1899, by his now famous hypothesis concerning the function of denitrifying bacteria in controlling the supply of available nitrogen in the ocean and, therefore, of plankton development. Brandt believed that most of the nitrogen compounds are brought into the sea from land by the rivers, streams, and drainage waters; without the activities of the denitrifying bacteria, nitrate concentration in the sea would soon reach a point where it would become injurious to living organisms. The more abundant plankton life in shallow waters, as compared with deep waters, was ascribed to the fact that, in the former, the available inorganic nutrients are distributed through a smaller volume of water, while in the deeper seas, the nutrients are distributed in much greater volumes of water. Plankton-rich seas were found to contain much more nitrate (and nitrite) than plankton-poor seas; a direct relation was thus shown to exist between the mass of plankton development and the amount of nitrate present. The conclusion was, therefore, reached that the abundance of nitrate controls the abundance of plankton. The tropical and sub-tropical seas are known to be comparatively poor in plankton, while the arctic seas are rich in plankton, especially in the summer; this phenomenon is just the opposite of that found in the case of land vegetation. This difference in the plankton development in the sea was explained by the difference in the concentration of available nitrogen compounds; the tropical climate favors active denitrification which results in the reduction of nitrate and the limitation of this most important plant nutrient in the sea.

These ideas resulted in an extensive study of the occurrence of denitrifying bacteria in the sea and their importance in marine processes. In 1902, Baur (2) reported the isolation of a denitrifying bacterium from the mud of an aquarium rich in nitrate; another form was isolated from the mud in the bay of Kiel. Gran (18) isolated from the sea, in 1901, several bacteria capable of reducing nitrate. He found that only at close proximity to shore, where organic residues are present in abundance and where large quantities of nitrate and nitrite are

brought in from shore, is there any possibility that true denitrifying bacteria play an important part in the destruction of nitrate. Gran concluded that Brandt's hypothesis is untenable.

Feitel (14) has shown in 1903 that denitrifying bacteria are present in great abundance in the sea, at all depths, but more often on the sea bottom or in the water near it, where organic matter is abundant. These bacteria were found not only close to land but also at a considerable distance from shore; when isolated in pure culture, they were capable of bringing about active denitrification, especially at low temperatures. Nathanson (37) demonstrated in 1906 the presence of denitrifying bacteria in the Gulf of Naples. In 1908, Kühl (29) found that marine mud brings about active denitrification. In 1909, Gräf (17) isolated from the waters of the Atlantic and Indian oceans 17 forms of denitrifying bacteria; this common occurrence of nitrate-reducing bacteria, taken together with the observations of the increase in nitrate concentration with an increase in the depth of water and the decrease of nitrate concentration with an increase in temperature, was believed to substantiate Brandt's hypothesis.

Issatchenko (25) found denitrifying bacteria in the North Polar Sea, capable of growing at very low temperatures (1–3° C.). He concluded that the bacteria reducing nitrate to nitrite or to ammonia are widely distributed in the sea at various depths and are represented there by numerous types, which are normal inhabitants of the sea. The true denitrifying bacteria, which are also widely distributed in the northern seas, were considered of doubtful importance in bringing about any appreciable losses of nitrogen in the sea. The fact that these bacteria are able to develop and reduce nitrate at low temperatures (12) and the fact that warm seas are also rich in nitrates (16) led Issatchenko to deny the validity of Brandt's hypothesis.

According to Gran (20), appreciable denitrification may take place only in enclosed bays and fjords, where large amounts of organic matter are present, considerable nitrate is brought in from land, and, due to limited circulation, the amount of oxygen is limited.

This brief summary of the literature bearing upon Brandt's hypothesis, concerning the importance of denitrifying bacteria in the cycle of life in the sea, points to the fact that the weight of the evidence bears out the conclusions of Gran and Issatchenko, namely, that admitting the existence of denitrifying bacteria in the sea, their rôle in marine processes is far from being established; certainly their part in controlling the nitrogen content of the sea and, therefore, of plankton development is shown to be of minor importance.

Another hypothesis based upon the activities of denitrifying bacteria

served to focus the attention of oceanographers on the rôle of these organisms in marine processes. Drew (13) attempted to correlate the activities of these bacteria with the process of calcium carbonate precipitation in the sea: nitrate-reducing bacteria decompose calcium salts of organic acids present in the sea; this results in the precipitation of the calcium as calcium carbonate. However, further studies on the occurrence of denitrifying bacteria in tropical seas and on the mechanism of calcium carbonate precipitation did not tend to confirm this hypothesis. Lipman (32) found that precipitation of $CaCO_3$ will take place in a suitable medium even in the absence of nitrate. Whatever the final explanation of the mechanism of calcium precipitation in the sea (32, 34, 3, 4), one is justified in concluding that the original hypothesis of Drew has not been substantiated by subsequent investigators. In order to bring about such extensive precipitation, large quantities of nitrate would have to be formed and still larger quantities of organic acids produced in the sea, two processes which have not been sufficiently established.

However, even if these two hypotheses concerning the importance of denitrifying bacteria in marine processes may be considered, for the present at least, as untenable, the fact remains that the sea harbors many bacteria capable of reducing nitrate to nitrite, to ammonia, and to atmospheric nitrogen (38, 35). Only a detailed study of the occurrence of these organisms in the sea and of their biochemical activities may enable one to interpret their true function in marine processes.

Experimental

Most of the media used by previous investigators for the growth of nitrate-reducing bacteria contained peptone (3, 34), which could serve both as a source of energy and of nitrogen. However, the use of an organic nitrogen compound for the growth of these bacteria tends to confuse the results obtained, since some of the products, such as ammonia, may either be due to the reduction of the nitrate or to the decomposition of the protein. In the following experiments, a synthetic medium was employed, with nitrate as the only source of nitrogen and with various carbohydrates or salts of organic acids as sources of energy.

In the first experiment, a study has been made of the relative abundance of nitrate-reducing bacteria in sea water and in marine mud. For this purpose the following medium was found to give good results:

Sea water	1000 cc.
Ca-acetate	10 grams
$NaNO_3$	1.0 gram
K_2HPO_4	0.5 gram
$FeSO_4 \cdot 7H_2O$	0.01 gram

Thirty-five-cc. portions of this medium and 10-gram portions of sand were placed in a series of large test tubes; these were plugged with cotton and sterilized, by heating at 70° C. for 4 hours. By the use of this medium and the dilution method, denitrifying bacteria, or organisms capable of reducing nitrate to gaseous forms of nitrogen, were found in dilutions of 1 cc. and 0.1 cc. of sea water, taken near shore, and in 0.0001 gram of mud.

The above method of sterilization proved to be unsatisfactory, since some of the controls contained bacterial spores. A study has, therefore, been made of the influence of sterilization of the medium upon the growth of nitrate-reducing bacteria. The results of this experiment are presented in Table V. Autoclaving the medium did not render it unfit for the development of these bacteria, hence one could feel fully justified in proceeding with this method of sterilization.

The above medium was used in the investigation of the distribution of nitrate-reducing bacteria in sea water and in mud in the Gulf of Maine. The results of this experiment, reported elsewhere (50),

TABLE V

Influence of sterilization of medium upon the development of denitrifying bacteria. Marine mud used for inoculation and cultures incubated for 3 days.

Dilution of mud	70° C., 2 hours*	100° C., 1 hour	15 lbs. pressure, 15 minutes	Filtration of culture through Seitz filter*
0.01	Gas	Gas	Gas	Gas
0.001	Gas	Gas	Gas	0
0.0001	Pellicle, no gas	Pellicle and gas	Gas	0
0.00001	0	Pellicle, no gas	Medium turbid, surface ring	0
Control	0	0	0	0

* Tubes and sand sterilized under pressure.

brought out the fact that bacteria reducing nitrate to nitrite are universally distributed in the sea. However, the occurrence in the open sea of bacteria reducing nitrate to atmospheric nitrogen is very limited. Only in the case of one station, with 0.1 gram of mud as the inoculum, was positive denitrification observed, when the nitrate was rapidly reduced to gaseous nitrogen. In none of the other seven stations studied and in none of the water samples obtained from the various depths could the presence of these organisms be demonstrated. The bacteria reducing nitrate to nitrite were found to be much more abundant in the mud than in the free water. Most of the nitrate-reducing organisms present in the water belonged to the non-spore-forming bacteria; how-

ever, several tubes inoculated with various dilutions of mud showed unmistakably the presence of spore-forming organisms. The actual amount of nitrate reduced in all the cultures reducing nitrate to nitrite was comparatively small.

In order to study the process of reduction of nitrate under controlled laboratory conditions, especially for the purpose of determining the influence of the energy source upon the reduction of nitrate to nitrite and to atmospheric nitrogen, the following experiment was carried out: 50-cc. portions of fresh sea water containing 50 mgm. of $NaNO_3$ were placed in a series of large tubes and 25-gram portions of sand added. Small portions of dry algal material, using *Fucus vesiculosus* or *Ulva lactuca,* were added to some of the tubes; some cultures, with and without nitrate, were used as controls. Some of the tubes were left without further inoculation, while some were inoculated with marine mud. All the cultures were incubated at room temperature and readings taken at the end of 5 and 9 days. The water used in this experiment was taken near shore at the Woods Hole harbor, while the mud was obtained off the shore of Gay Head.

The results presented in Table VI show that algal material can be used readily as a source of energy for the reduction of nitrate by the bacteria living in the sea water and in marine mud. There were found several marked differences, however, between the different cultures, depending on the nature of the algal material and on the kind of inoculum. In the sea water medium, the organic constituents of the *Ulva* were used readily as sources of energy for the reduction of the nitrate, as shown by the rapid reduction of the nitrate to nitrite and to gaseous nitrogen within 5 days; after 9 days incubation, all the nitrate and nitrite have completely disappeared, due to their reduction to atmospheric nitrogen. When *Fucus* was added to the sea water, the reduction of the nitrate was much slower, no gas being formed and considerable nitrate and nitrite being still left after 9 days incubation. The sea water medium containing nitrate only and no additional energy source showed no trace of reduction of nitrate, either to nitrite or to gaseous nitrogen.

The cultures heavily inoculated with mud gave a totally different type of nitrate reduction. When the *Ulva* material was added to the medium, both the nitrate and nitrite disappeared in 9 days, with active gas evolution, even more rapidly than in the cultures of sea water alone. In the case of the cultures receiving the *Fucus* the reduction of the nitrate was so rapid that, within 5 days, all the nitrate and nitrite disappeared completely from the culture, accompanied by abundant gas evolution. The difference in the influence of the *Fucus* material in the

cultures inoculated with water and with mud is due largely to the fact that, as suggested previously, marine mud probably contains bacteria capable of attacking the specific carbohydrates of the *Fucus*, while such bacteria are absent in the sea water itself. The mud-inoculated cultures receiving no algal material also gave active reduction of nitrate to nitrite and even to atmospheric nitrogen; in this case the small amount of available organic matter in the mud (46) was probably used

TABLE VI

Utilization of marine algæ as sources of energy for bacteria reducing nitrate.

Tr = trace of nitrite or nitrate; 0 indicates negative test; + = positive nitrite or nitrate test; ++ and +++ = considerable nitrite or nitrate present; +++++ = maximum nitrite formation.

Source of algal material	Mud inoculum	Growth of bacteria	Gas		Nitrite		Nitrate	
			5 days	9 days	5 days	9 days	5 days	9 days
Sea water alone.....		0	0	0	0	0	0	0
Sea water and nitrate.....		0	0	0	0	0	++	++
Sea water, nitrate, and Ulva.....		Turbidity in culture	+	0	+++++	0	++	tr
Sea water, nitrate, and Fucus.....		Turbidity and pellicle	0	0	+++	+++++	++	+
Sea water alone	+	0	0	0	0	+	0	0
Sea water and nitrate.....	+	Turbidity	+	0	++	++	++	++
Sea water, nitrate, and Ulva.....	+	Turbidity	+	+	+	0	+	0
Sea water, nitrate, and Fucus.....	+	Pellicle	+	+	0	0	0	0

as a source of energy. The traces of nitrite found in the culture which had not received any nitrate but which was inoculated with mud are possibly due to the formation of nitrite by the oxidation of the ammonia which is formed in the decomposition of the organic nitrogenous complexes in the mud.

A study of the results of the two experiments, namely, (1) those of the cruise of the "Atlantis," which pointed to the presence in the sea of bacteria capable of reducing nitrate chiefly to nitrite, and (2) those

emphasizing the importance of the specific energy source for the activities of the nitrate-reducing bacteria, will justify one in drawing certain definite conclusions. The marine algæ and members of the phytoplankton live and synthesize their cell substance in the surface layers of the water, or in the zone of maximum photosynthetic activities. As a result of these synthetic processes, the plants consume the nitrate present in the particular layers of water in the sea. The bacteria present in the water or attached to the algæ or the phytoplankton feed upon certain secretion products of these marine plants or upon the plant detritus, after the plants die. Since these algal and diatom substances are either free from nitrogen, as in the case of some of the secretion products, or contain only very limited amounts of this element, the bacteria decomposing them, unless they are nitrogen-fixing organisms, have to draw upon the available nitrogen present in the surrounding medium, namely in the sea. This results in the consumption of a certain amount of nitrate by the bacteria. As a result of these two processes, namely phytoplankton development and bacterial consumption of the algal products, the nitrate tends to disappear in the surface layers of water in the sea.

Among the bacteria attacking the algal and diatom products and residues in the surface layers of the water are present organisms which not only consume the nitrate and transform it into organic nitrogenous compounds, as constituents of the bacterial cell substance, but which also reduce the nitrate. Such reduction will take place only in the presence of an excess of nitrate, above that consumed by the algæ and diatoms and the heterotrophic marine bacteria. This reduction is carried largely to the nitrite stage, but not to atmospheric nitrogen. One would, therefore, expect that where active plant growth takes place in the sea nitrites should be present rather than nitrates. This does not indicate any loss of nitrogen from the sea but merely that at a certain level below the surface of the water, conditions are favorable to the reduction of the nitrate to nitrite. The latter can, of course, be used by the growing algæ and diatoms as a source of nitrogen just as well as the nitrate itself.

The marine bottom presents a totally different bacteriological system from that of the water itself. Not only does it harbor an extensive bacterial population capable of reducing nitrate to nitrite but it may also contain true denitrifying bacteria. However, the occurrence of such bacteria in the mud does not at all indicate that the process of complete reduction of nitrate to atmospheric nitrogen is very pronounced even at the sea bottom. Here also, certain controls prevent the rapid losses of nitrogen in a combined form which is so essential

for the life of the plants in the sea. In order to reduce the nitrate (or nitrite) to atmospheric nitrogen, the denitrifying bacteria must have a source of available energy; but the organic matter of the sea bottom, or the marine humus, can be used only to a limited extent as this source of energy. This marine humus contains carbon and nitrogen in such proportions (46) that its decomposition will give rise to available nitrogen in the form of ammonia, which is used as a substrate for the bacteria producing nitrate, through the nitrite stage. The only existing possibility for denitrification to take place is in the very surface layer of the marine bottom where incompletely decomposed plant residues may offer a source of available energy for the denitrifying bacteria. It is doubtful whether even under these conditions the nitrate-consuming bacteria, decomposing these residues, are not more predominant and more active than the nitrate-destroying bacteria.

Occurrence of Nitrogen-Fixing Bacteria in the Sea

Historical

Although the ocean is usually considered to be a large reservoir of combined nitrogen, the question as to the origin of this nitrogen has frequently been raised. Some investigators believed that it is a direct result of electric discharges, while others looked to the land as the sole source of this nitrogen; however, the belief expressed by some that bacteria are responsible for the presence of combined nitrogen in the sea has been gaining ground. There is no doubt that all these three theories have some justification. The presence of a considerable amount of nitrogen in marine mud (46), especially not far from land, points to the latter as a possible source of at least some of the nitrogen present in the sea; whether this nitrogen is readily available for the nutrition of marine algæ and plankton is another question, and one which is still little understood.

The function of the nitrogen-fixing bacteria in increasing the supply of combined nitrogen in the sea is difficult to determine, the results obtained under laboratory conditions not lending themselves readily to a direct application to natural marine processes. While the importance of the bacteria concerned in the fixation of atmospheric nitrogen on land is well established, the available information about the occurrence and activities of these bacteria in the sea is still very elementary. When one considers further the fact that, of the two groups of nitrogen-fixing bacteria on land, the function of only the symbiotic organisms, namely root-nodule bacteria, is well established, while the importance of the non-symbiotic bacteria is still in dispute, and that it is the latter

group which is found in the sea, one can readily understand the reason for the lack of sufficient knowledge concerning the activities of these bacteria in the sea.

Benecke and Keutner (7) were the first to demonstrate that both the ærobic *Azotobacter* and the anærobic *Clostridium pastorianum* are found in the sea, the former occurring largely on the plankton, while the latter is present chiefly in the lower layers of water and in the sea bottom. When an attempt was made to isolate these organisms, they were found to be always accompanied by other bacteria, largely two spore-forming types. Keutner (28) concluded in 1905 that nitrogen-fixing bacteria are normal inhabitants of the sea, having been demonstrated in the North Sea, in the Indian Ocean, on the African coast, and in the Malay Archipelago. The amount of nitrogen fixed per culture containing 4 grams of glucose in 200 cc. of water varied from 5.7 to 16.0 milligrams, in the presence of calcium carbonate, and from 0.9 to 9.0 milligrams in its absence.

Keding (27) found *Azotobacter* to be present in the slimy substance formed on the surface of various marine algæ; he believed that the strains of this organism found in the sea are identical to those present on land. Nathanson (37), however, could not find *Azotobacter* in the Gulf of Naples; as a result of this, he was inclined to conclude that the occurrence of this organism in the sea is merely a matter of infection with land water. Benecke (6) repeated this work and had no difficulty in establishing the common occurrence of this organism in the Gulf of Naples; the negative results obtained by Nathanson were due entirely to improper methods of study. As is usually the case, negative results are frequently no proof at all that a specific organism is absent in the sea, but may be due to a lack of a proper technique or to unfavorable conditions of culture. Typical *Azotobacter* cultures were isolated by Benecke from mud taken at a depth of 20 to 100 meters.

The fact that *Azotobacter* occurs abundantly on marine algæ led Reinke (40, 41) to suggest that one deals here with a case of symbiosis, similar to that of leguminous plants, the algæ supplying *Azotobacter* with carbohydrates as sources of energy and using the nitrogen fixed by the latter. Issatchenko (24, 25) also demonstrated the presence of typical strains of *Azotobacter* and *Clostridium* in media inoculated with some fresh *Fucus*. When the medium was free from salt no development took place, tending to indicate that one deals here with organisms specifically adapted to sea water. The presence of both *Azotobacter* and *Clostridium* was established by Bavendamm (4) in the calcareous mud of various parts of the Bahama region, near Williams Island.

These results lead one to the conclusion that nitrogen-fixing bac-

teria are present in the sea, especially in the bottom material and in connection with the phytoplankton and marine algæ. Since these bacteria require a source of energy for their development and since the marine humus is not available for this purpose, one would naturally expect to find them in places where energy is made available either through the growth of the chlorophyl-bearing forms of life or after their death.

Experimental

The following experiments deal largely with the methods to be used for demonstrating the presence of nitrogen-fixing bacteria in the sea. In all cases the organisms must be allowed to develop on a favorable enrichment medium, containing an available source of energy and the necessary minerals which are either lacking in sea water or are present there in limited amounts. A presumptive test for the presence of non-symbiotic nitrogen-fixing bacteria in the enriched culture is the demonstration, by means of microscopic preparations, of the characteristic cells of the ærobic *Azotobacter* or the anærobic *Clostridium pastorianum*. Confirmative tests are made by determining the increase in total nitrogen content of the medium; for full confirmation, however, the organism must be isolated in pure culture and its ability to increase the total nitrogen content of an artificial medium determined.

The first experiment was largely of a preliminary nature, namely on the influence of the energy source upon the development of nitrogen-fixing bacteria in sea water media. One hundred-cc. portions of fresh sea water containing 0.05 per cent of K_2HPO_4 were placed in a series of flasks and 1 per cent quantities of various energy sources added. Some of the flasks received 40 grams of moist sand and others did not. When glucose and mannitol were used as sources of energy, abundant gas formation and butyric acid development took place, within 4–10 days in the flasks containing sand; this pointed to the development of anærobic butyric acid bacteria. The sand-free flasks gave some growth, which was not typical, however, of nitrogen-fixing bacteria. In view of the fact that no $CaCO_3$ was added to the medium and that the reaction was changed to pH 5.4–4.4, due to acid production, the lack of *Azotobacter* development could be readily understood. When calcium acetate was used as a source of energy, active development of the anærobic *Clostridium* was accompanied by the growth of *Azotobacter;* the final reaction of this medium was pH 7.4. Sterilization of the medium followed by inoculation with fresh sea water did not influence the development of the nitrogen-fixing organisms.

In view of the importance of iron in the process of fixation of nitrogen by *Azotobacter,* an iron-ligno protein compound (49) was also

added. The final basic medium adopted for these investigations consisted of:

1000 cc. sea water
0.5 gram K_2HPO_4
0.5 gram iron-ligno-proteinate
20 grams glucose, mannitol, or Ca acetate.

The purpose of the next experiment was to establish the ability of the mixed bacterial population of the sea to fix nitrogen. One hundred cubic centimeter portions of the above medium, prepared from fresh sea water, were placed in a series of 250-cc. Erlenmeyer flasks. Some of the flasks received 40-gram portions of sand, 2–3 grams of $CaCO_3$ and 2 grams of the energy source; some flasks received only $CaCO_3$ and the energy source, while some did not receive any $CaCO_3$. The cultures were incubated for 32 days and the nitrogen determinations made.

The results presented in Table VII prove that sea water contains active nitrogen-fixing organisms. The presence of sand is not necessary to bring about their optimum activities. Glucose and mannitol were found to be usually better sources of energy than Ca acetate. The addition of $CaCO_3$ is necessary, when sugars are used, to neutralize the

TABLE VII

Nitrogen fixation in sea water with and without sand, with different carbon sources. Two grams of energy source used per culture. Cultures were incubated 32 days.

Sand present	Energy source	$CaCO_3$ added	Gas formation	Nitrogen fixed per culture
				mg.
−	Glucose		0	11.2
−	Glucose	+	0	12.5
−	Mannitol		+	0.2
−	Mannitol	+	+	13.8
−	Ca acetate		+	0.9
−	Ca acetate	+	Trace	2.0
+	Glucose		+	3.7
+	Glucose	+	0	8.2
+	Mannitol		+	4.8
+	Mannitol	+	0	8.6
+	Ca acetate		0	3.1
+	Ca acetate	+	0	4.6

acidity produced by the anærobic organisms; the presence and activities of these are indicated by the abundant gas evolution. The crude cultures fixed appreciable quantities of nitrogen. The fixation of nitrogen was greatest in the presence of $CaCO_3$, except in the case of those cul-

tures where lack of gas evolution pointed to the unfavorable conditions for the development of the anærobic bacteria; a good pellicle development of *Azotobacter* and accompanying forms always indicated abundant fixation of nitrogen.

In order to test the presence and distribution of nitrogen-fixing bacteria in the sea, two types of media were used, one favorable for the development of anærobic bacteria and one for the growth of ærobic organisms: 1. The medium previously described was prepared, and 35-cc. portions, together with 10 grams of dry washed sand, were placed in large test tubes and sterilized for 15 minutes at 15 lbs. pressure; this medium was particularly favorable for demonstrating the presence of the anærobic *Clostridium* in sea water and in sea bottom; the relative abundance of this organism was determined by the dilution procedure. 2. The silica-gel plate was utilized for the purpose of demonstrating the presence of ærobic nitrogen-fixing bacteria in the sea.

The plates containing the gel (47) are placed in flowing tap water for 12–16 hours, to remove excess of chloride; they are then removed from the tap water and immersed in freshly boiled and cooled sea water, for 5–10 minutes. The excess of water is drained off and carefully dried off and the plates covered with sterile tops. A nutrient solution [4] is prepared so that when 2 cc. of this solution is added to a 100-cc. portion of silica gel, using a plate 15 cm. in diameter, the final medium will contain 2 per cent of the carbon source. Distilled water is used for the preparation of the nutrient solution. The plates are then exposed in a drying oven kept at 60° C. for a few minutes, until the excess of water has been removed. A thin layer of sterile $CaCO_3$ is powdered on the surface of the medium, the plates are covered with sterile tops, and are ready for inoculation. Special care must be taken in the preparation of this medium, since an excess of salt or too thin a layer of medium will result in the rapid drying out of the gel and will prevent bacterial development.

The cruise of the "Atlantis" in August, 1932, to the Gulf of Maine and George's bank (50) presented a good opportunity for determining the presence of ærobic and anærobic nitrogen-fixing bacteria on the high seas, both in the sea water and in the mud. A series of tubes with sterile liquid medium and a series of plates with the silica-gel medium were taken aboard ship and inoculated with water and mud from the various stations, immediately after the samples were taken. It was demonstrated in a previous contribution (50) that there is a definite parallelism between the total numbers of bacteria in the water, as determined by the plate method, and the number of plankton organisms;

[4] This solution also contained 0.05 per cent K_2HPO_4 and 0.05 per cent iron lignoprotein complex.

this was especially true of the phytoplankton. Whether the occurrence of *Azotobacter* in the sea has also a certain relation to the phytoplankton still remains to be determined. At the time of the cruise, there was comparatively little phytoplankton in the water. This may offer the explanation for the fact that not all the stations, especially the free water, gave evidence of *Azotobacter* growth. It may also be due to the fact that the silica gel medium used in this experiment was not quite favorable for the growth of the organism, as found later when the details of the method were better understood.

In the case of one station (1329), the surface layer of the marine mud gave an excellent growth of the typical *Azotobacter chroöcoccum*, using the silica gel plate. The liquid cultures showed marked differences, when water and mud were used as inocula. When 1 cc. of surface water was added to the medium, good pellicle development took place; the pellicle contained typical *Azotobacter* cells, which were also accompanied by various other bacteria. In the case of one station

TABLE VIII

Fixation of nitrogen by crude culture of Azotobacter chroöcoccum. Two-gram energy source.

Energy source	Organism	Medium	Incubation	Nitrogen fixed per culture
			days	*mg.*
Glucose	Control	Silica plate	21	0.1
Glucose	Azotobacter	Silica plate	21	5.4
Mannitol	Azotobacter	Liquid medium	15	6.4
Glucose	Azotobacter	Liquid medium	17	4.8

(1331), samples of water were taken, by means of sterile glass tubes, at the surface of the water and at depths of 30, 50, 100, and 215 meters. Only the surface and the 50 meter samples gave pellicle formation on the liquid medium; the other samples gave no signs of growth. Very few of the samples of free sea water, obtained from the different stations, brought about any gas formation or butyric acid production in the liquid medium, thus proving the absence of the anærobic *Clostridium* in the free water.

The liquid media inoculated with the mud samples gave abundant gas formation, the medium itself becoming decidedly turbid. A microscopic examination of the cultures showed the presence of *Clostridium* cells, especially when glucose was used as a source of energy. This organism was always accompanied by other bacteria, usually small and large rods, some of which were spore-forming organisms.

These results suggest that anærobic nitrogen-fixing bacteria of the

Clostridium pastorianum type are universally distributed in the sea bottom, but are present only to a very limited extent in the sea water. The ærobic *Azotobacter* is also found in the sea, but much less frequently than the anærobic *Clostridium*. Whether *Azotobacter* is less abundant in the sea or whether the media used for demonstrating its presence were not always sufficiently favorable for its development remains to be determined. Although there is no doubt that *Clostridium* finds its natural medium in the sea bottom, it still remains to be established whether *Azotobacter* is normally present in the sea water, in the plankton, on the surface of algal growth, or in the sea bottom. Benecke and Keutner, Issatchenko, and others found this organism on the surface of marine algæ and suggested, therefore, its symbiotic existence with the latter; however, its presence on the bottom of the sea suggests also the possibility of its free existence, whereby the remains of algal and plankton growth are used as sources of energy.

Various other bacteria were encountered in the nitrogen-free media, either accompanying *Clostridium* and *Azotobacter,* or even without these organisms; they consisted of different cocci and small rods, occasionally thread-like forms. Whether these bacteria are able to fix nitrogen or whether they play any part in this process at all still remains to be determined.

In order to demonstrate the ability of the culture of *Az. chroöcoccum* obtained from the bottom mud of Station 1329, by the use of the glucose-silica-gel plate, to fix atmospheric nitrogen, the total nitrogen content of the gel in which this organism was growing was compared with that of a control plate. The crude culture of this organism was also inoculated into a 100-cc. portion of liquid medium containing 2 per cent of glucose or mannitol, incubated for 15–17 days and the amount of nitrogen fixed determined (Table VIII). The results obtained prove beyond any doubt the ability of the *Azotobacter* found in the sea bottom to fix considerable quantities of nitrogen in sea water media, in the presence of available sources of energy.

The *Azotobacter* was later isolated free from the accompanying bacteria, as shown in Fig. 3. However, in view of the fact that the nitrogen-fixing organisms do not live in the sea in the form of pure cultures, but are present there as mixed populations, a knowledge of their ability to fix nitrogen in such a mixture is more important than that of a pure culture.

Figures 1 to 8 show a group of preparations of bacteria isolated from the nitrogen-fixing cultures in which sea water and bottom material of the Gulf of Maine were used. The *Azotobacter* produced on the silica gel plate a typical growth of *Az. chroöcoccum,* forming at first

a yellow pigment which later turned deep brown to black. The *Clostridium* produced abundant gas from sugars in liquid media. The ability of the three small bacterial forms shown in Figs. 6, 7, and 8 to fix nitrogen has not been determined. They are common types which develop on nitrogen-free media inoculated with sea water or with sea bottom material.

Although the results presented here are to be considered as preliminary in nature, they are quite sufficient to bring out the fact that nitrogen-fixing bacteria, both of the ærobic and anærobic types, are commonly found in the sea. There is no doubt that in the presence of an available source of energy and other nutrients, such as available iron and phosphate, they become active in the sea and are able to fix atmospheric nitrogen.

A study of the relation of these bacteria to the phytoplankton and plankton residues on the sea bottom, the natural sources from which they derive their energy, would necessitate a study of the occurrence of *Azotobacter* at certain stations throughout the year. This, as well as further study of the relation of the nitrogen-fixing bacteria to the available nutrients in the sea, changing conditions, season of year, depth, distance from shore, etc., are still problems awaiting further investigation. For this purpose, the silica gel plate method seems to be ideal for the determination of the occurrence of *Azotobacter*, while the liquid-sand culture in the tube is very convenient for studying the abundance of *Clostridium*.

Summary

1. A preliminary study has been made of the occurrence and activities in the sea of bacteria concerned in the cycle of nitrogen in marine life.

EXPLANATION OF PLATE I

Fig. 1. Mixed culture of *Azotobacter* and accompanying bacteria. Mud of Station 1329 inoculated on silica gel plate. × 1031.

Fig. 2. Culture of *Azotobacter* on liquid media, isolated from sea water, accompanied by short rod-shaped bacteria. × 1031.

Fig. 3. Pure culture of *Azotobacter* isolated from sea water. × 1031.

Fig. 4. Culture of *Azotobacter* isolated from mud of Station 1329 on silica-gel media and cultivated on nitrogen-free glucose media, showing granulation. × 1031.

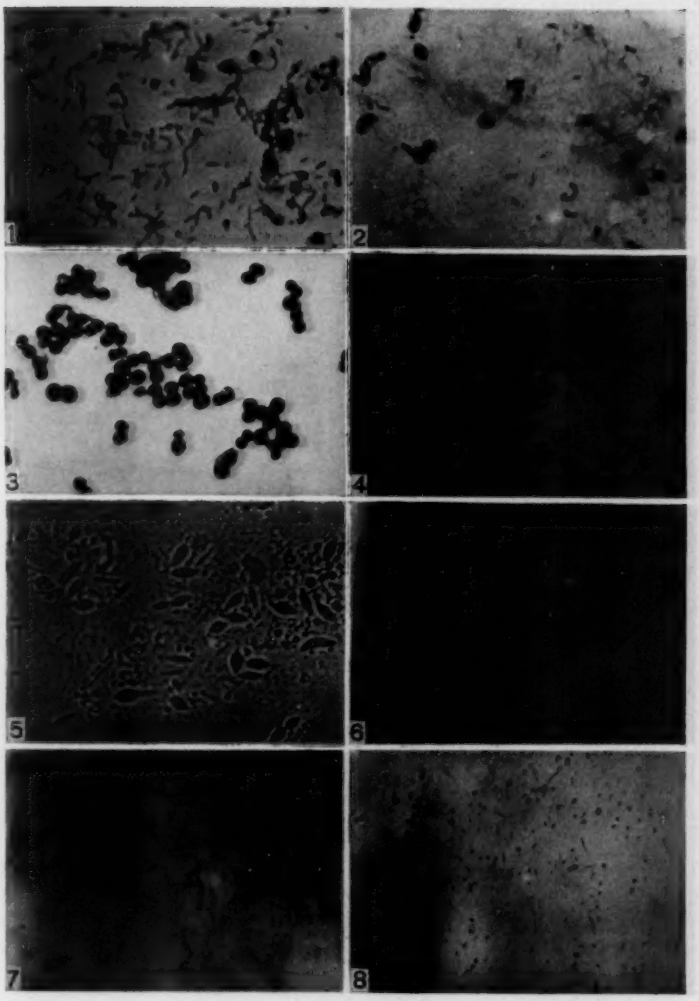
Fig. 5. Culture of *Clostridium* isolated from marine mud. Culture impure, unstained preparation. × 1031.

Fig. 6. Thread-forming bacteria growing in nitrogen-free glucose media inoculated with marine mud. × 1031.

Fig. 7. Spore-forming rod-shaped bacteria growing on nitrogen-free media. × 1031.

Fig. 8. Spore-forming rod-shaped bacteria isolated from surface water in the Gulf of Maine (Station 1331) and grown on nitrogen-free mannitol media. × 1031.

PLATE I



2. Sea water, especially in the upper layers, is practically free from nitrifying bacteria or contains only very few cells. The activities of these organisms are limited almost entirely to the sea bottom.

3. In order to demonstrate the presence of these organisms even in the bottom material, it is essential to use a medium containing a layer of sand or mud, so as to make conditions favorable for their development.

4. It is comparatively easy to demonstrate the presence in the sea bottom of bacteria oxidizing ammonium salts to nitrite. It is somewhat more difficult to establish the presence of nitrate-forming bacteria; this can be done, however, if the sensitiveness of these organisms to the laboratory conditions is taken into consideration, especially nutrient concentration and period of incubation.

5. Sea water and sea bottom contain an abundance of nitrate-reducing bacteria. Most of these organisms, especially those present in the water, are able, however, to reduce the nitrate only to nitrite, but not to atmospheric nitrogen.

6. Bacteria that bring about complete reduction of the nitrate are present largely in the sea bottom. In order to reduce the nitrate, an available source of energy must be present. The marine humus cannot be used to any great extent as such a source of energy. The plankton residues are used largely by those bacteria which consume the nitrate and transform it into organic nitrogenous compounds.

7. The conclusion is reached that, even if denitrifying bacteria are present in the sea, their activities are so limited under marine conditions as to render them in most instances of little importance in limiting the nitrate supply of the sea.

8. The sea contains an abundant population of nitrogen-fixing bacteria. The ærobic *Azotobacter* is largely found in the water and in the surface layer of the marine bottom, while the anærobic *Clostridium* is present in the sea bottom.

9. In the presence of a favorable source of energy, the marine nitrogen-fixing bacteria are capable of fixing appreciable quantities of nitrogen. It still remains to be determined, however, to what extent this process actually takes place in the sea.

10. The bacteria concerned in the transformation of nitrogen can be tentatively distributed among the plankton and the sea bottom, the two major seats of bacterial activities in the sea, as follows: the nitrifying bacteria live largely on the sea bottom; of the nitrate-reducing bacteria, those forms which reduce nitrate to nitrite live both in the water and in the bottom, while the true denitrifying bacteria live largely on the bottom; of the nitrogen-fixing bacteria, the ærobic forms live both in the

water and on the bottom, while the anærobic forms live largely in the bottom material.

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LIGHT INTENSITY AND THE EXTENT OF ACTIVITY OF LOCOMOTOR MUSCLES AS OPPOSED TO CILIA

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I

The effect of light on animal behavior has attracted the attention of biologists for many years. One phase of the general problem, namely the photokinetic effect of light, has been studied to a less extent than orientation and the factors determining the phototropic condition of organisms and many questions regarding this phase of photic behavior remain to be answered. The first and most important of these is whether or not intensity of illumination does actually influence the speed at which an animal moves toward or away from a source of illumination. Some of the evidence has already been reviewed by the author (Welsh, 1932*a*). In the same paper and in additional work it has been shown that in a light-sensitive organism, which moves by means of muscles, the extent of muscular activity bears a very definite relationship to intensity of illumination. This is not in agreement with work of Mast (1923), and Dolley (1917), but does agree with the results of most investigators and seems reasonable in the light of what we know regarding the response of the receptor-muscle system to light. It is not as evident that light intensity has a similar effect on organisms which move by means of cilia or flagellæ. Observations on such forms indicate that there is no effect of luminous intensity on speed of progression. Directional light, however, does influence orientation in these animals and it is difficult to determine whether slight changes in rate are due to deviations from a straight path or to changes in amplitude and frequency of ciliary movement. It seemed desirable to search for forms which move by means of cilia and which, after primary orientation, proceed in a straight line. A study of such forms should indicate whether or not their speed of progression is a function of the intensity of illumination.

In the course of some work at the Woods Hole Oceanographic Institution there was an opportunity for such an investigation and a

[1] Contribution No. 21 from the Woods Hole Oceanographic Institution.

survey was made of the effect of light intensity on the activity of a number of marine invertebrates. The results indicate that the extent of ciliary activity is not a function of the amount of light energy reaching the organism as is true of the activity of locomotor muscles.

II

Among the animals used in the investigation were two species of small marine turbellarians, one of which was identified as *Plagiostomum* sp.; the other has not yet been identified. These were obtained largely from *Ulva* where they were found moving over the surface of the alga. They both orient by means of muscles but after primary orientation cilia alone seem to be concerned in locomotion. One important difference made these animals particularly valuable for purposes of comparison. *Plagiostomum* tends to be free-swimming and the path through which it moves is not straight, while the unidentified form always moved in contact with the substrate and its path was a nearly perfect straight line. *Columbella lunata,* a small gastropod, also moves by means of cilia. Orientation again involves muscles but they appear to play no part in forward locomotion. *Arenicola* larvæ were also used but the spiralling of this form made it difficult to distinguish between orientation effects and any change in extent of ciliary activity.

Among the animals observed which move by means of appendages operated by muscles were larvæ of *Pinnotheres maculatus, Hippa talpoida,* and stomatopod larvæ, probably of *Squilla empusa.* The copepod *Centropages typicus* and small specimens of *Limulus polyphemus* were also used. All of these forms, at the age used, were positive to light with the exception of *Limulus* which was negative.

The experiments were carried on under carefully controlled conditions. The animals were placed individually in a glass trough, 30 cm. in length, with the bottom marked in centimeters. This trough was partially submerged in a water bath maintained at 21.3° C. Light reached the animals through windows in opposite sides of the tank. The light sources were two ribbon filament lamps arranged to give an even illumination of nearly parallel rays in the mid-region of the trough, the illumination from one lamp being 1500 foot candles at this point and from the other 7.5 foot candles. These intensities were selected after numerous tests on different species had shown that the intensity of 1500 foot candles was higher than that necessary to elicit a maximum speed, while 7.5 foot candles fell within the range of intensities to which these same organisms showed a change in speed of progression (*e.g. Pinnotheres* larvæ, Welsh, 1932*a*; *Unionicola,* Welsh, 1932*b*).

Both lamps were operated from a three-way switch so that one was turned off as the other was turned on.

The animal under observation was adapted to the temperature of the sea water in which it was placed and then timed while traversing the middle 10 cm. of the trough. Ten trials were made for a given animal at each of the two light intensities, and the times were averaged. The results are shown in the following section.

III

For an analysis of the findings the animals may be divided into three groups according to their methods of locomotion and the extent to which orientation may affect the apparent velocity of forward pro-

TABLE I

The effect of light intensity on the speed of forward progression of animals moving by means of locomotor appendages operated by muscles.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Pinnotheres larva	6.6	±0.13	14.1	±0.47
Hippa larva	4.5	±0.06	11.1	±0.16
Squilla larva	4.9	±0.45	11.3	±0.66
Limulus	5.4	±0.05	7.4	±0.25
Centropages	4.4	±0.22	8.5	±0.77

gression. We may place in the first group those animals which move by means of locomotor appendages operated by muscles; in the second group animals moving by means of cilia and exhibiting considerable variation from a straight path in directional light; and in the third group animals moving by means of cilia and progressing in a straight line in directional light.

Table I gives the average times, with their probable errors, for the first group. The results indicate that light intensity greatly influences the speed, as the times for travelling 10 cm. at the lower intensity are over twice as great as those at the higher intensity in all instances but one. This indicates that the frequency and amplitude of movement of the locomotor appendages must increase with an increase in intensity, as is true for *Unionicola* (Welsh, 1932*b*). This may be accounted for by assuming a more rapid decomposition of photo-sensitive material in the photoreceptor as shown by Hecht (1920), followed by a greater

frequency of discharge over sensory tracts from the eye (Hartline and Graham, 1932), and finally more muscle fibers of the locomotor appendages being stimulated by discharge of motor nerves. Throughout this system thresholds of receptors, conductors, and effectors may be involved as well as frequency of discharge of the nerves, and it is readily apparent that the amount of light energy reaching the organism might well be expressed by the degree of activity of locomotor structures, and hence the speed at which the animal travels.

Table II gives the times for traversing 10 cm. for three different animals belonging to a species of *Plagiostomum*, and for a larva of the lug-worm *Arenicola*. In both forms cilia are involved in forward locomotion but there are considerable deviations from a straight path

TABLE II

The effect of light intensity on the speed of forward progression of animals moving by means of cilia and exhibiting considerable variation from a straight path in directional light.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Plagiostomum (1)	52.6	±2.83	61.8	±2.75
Plagiostomum (2)	33.9	±0.84	37.7	±0.65
Plagiostomum (3)	35.8	±1.80	42.6	±1.03
Arenicola larva	78.5	±1.02	123.5	± 5.6

even in progression toward a light of high intensity. The times for travelling 10 cm. at the lower intensity are greater than those for 1500 foot candles, but in the case of *Plagiostomum* the difference is not large and there is no agreement between the average times and their probable errors as is shown in Table I. The probable error in each instance is less for the lower intensity than for the higher. It seems in the case of *Plagiostomum* that the difference in times at the two intensities might be accounted for by the deviations from a straight path which are greater at the lower of the two intensities. These deviations involve muscular bending of the body which helps in no apparent way with forward progression.

In the case of the *Arenicola* larva the difference in times at the two intensities is proportionally greater than that for *Plagiostomum* but the deviations from a straight path were more pronounced. At the lower intensity the degree of spiralling increased and also an up and down movement was more noticeable.

Now if we examine the results from another turbellarian and from *Columbella lunata* (Table III), it is seen that intensity of illumination has no effect on the speed of progression. In these forms, as stated previously, muscles play an important part in orientation and the angle of turning bears a definite relationship to the luminous intensity. After orientation, however, the movement toward the light was a smooth, gliding one, and the deviations from a straight path were negligible. While there was considerable variation in the speed of different animals of the same species, the speed of any particular specimen was quite uniform, and this was true irrespective of light intensity.

These results would indicate that the beat of cilia is not affected by light intensity as is the frequency and amplitude of movement of loco-

TABLE III

The effect of light intensity on the speed of forward progression of animals moving by means of cilia and progressing in a straight line in directional light.

Animal	I = 1500 ft. candles		I = 7.5 ft. candles	
	Time for travelling 10 cm.	P. E.	Time for travelling 10 cm.	P. E.
	seconds		*seconds*	
Turbellarian sp. ? (1)	49.5	±0.57	50.1	±0.50
Turbellarian sp. ? (2)	89.1	±2.63	89.6	±2.48
Turbellarian sp. ? (3)	37.9	±0.28	37.4	±0.44
Columbella	80.2	±2.15	81.2	±2.42

motor muscles. These two types of effectors are quite different in their behavior to stimuli other than light, and there is no known mechanism whereby the amount of light energy reaching the photoreceptors might be realized by the cilia except in an indirect way.

IV

The results obtained on animals progressing by means of cilia are in agreement with those of Holmes (1903), and Mast and Gover (1922) obtained on flagellated forms, but they indicate that the laws governing these forms cannot be applied to forms such as the arthropods which move by means of appendages operated by muscles.

In the case of planaria there is an apparent effect of light on speed of progression (Parker and Burnett, 1900; Pearl, 1903; Walter, 1907), but in these animals, according to Stringer (1917), locomotion is essentially a muscular act in which cilia play no necessary part. Evi-

dently only in the smaller turbellarians do cilia function as important locomotor structures. This is probably also true of the gastropods.

The observations of Umeda (1927), Wharton (1931), and Lucas (1933), as reported by Lucas, concerning the effect of light on ciliated epithelium, are of interest in this consideration although they are somewhat contradictory. Lucas (1933) found that in ciliated epithelium from the frog, quiescent cilia were sometimes made active by illumination. He reports in the same paper that Umeda obtained similar results from illumination with light of long wave lengths. Wharton (1931), however, in studying cilia of the tracheae, found that light had an apparent inhibitory effect on the beat of the cilia. As the present writer is not familiar with the experimental technique used by these investigators it is not possible to comment on the results. It is difficult to conceive of light, dissociated entirely from heat, having a direct effect on the activity of cilia of ciliated epithelium although such an effect may be possible. This problem would seem to be worthy of further investigation.

It seems safe to conclude from the evidence at hand that the activity of locomotor muscles of certain animals, and hence the speed of progression of these forms, is a definite function of the intensity of illumination over a given range, while the activity of cilia, and speed of animals moving by means of cilia bears no such relation to light intensity.

Summary

The results obtained from a study of the effect of intensity of illumination on the speed of progression of a number of marine invertebrates indicate that the activity of locomotor appendages, operated by muscles, is a function of the luminous intensity and that the animals move faster in light of high intensity than in light of low intensity. This, however, is not true of animals moving by means of cilia, for such animals, provided they move in a straight line in directional light, show no change in speed and hence no change in ciliary beat as the light intensity is varied.

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CONCHOPHTHIRIUS CARYOCLADA SP. NOV. (PROTOZOA, CILIATA)

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During the month of August, 1932, the writer noticed a large holotrichous ciliate in the mantle cavity of the edible clam, *Siliqua patula*, taken from the sandy beach at Seaside, Oregon. After studying its general morphology it was decided that it was a hitherto undescribed species of the genus *Conchophthirius*. Because of its characteristically branched macronucleus it will be referred to as *Conchophthirius caryoclada*.

The methods used in this study are, in general, those described in an earlier work on this genus (Kidder, 1933*a*). Observations were made on both living and stained material.

The clams were found to be only lightly infected and while from the majority two or more ciliates were obtained the material was limited. No cases of binary fission or conjugation were found.

A study of the general morphology of *C. caryoclada* shows it to be nearly oval as viewed from the dorsal or ventral side. The size ranges from 140 μ to 250 μ in length and from 90 μ to 160 μ in width. In lateral view it is extremely thin and leaf-like, the dorso-ventral axis not exceeding one-eighth that of the lateral axis. The body is covered with fine cilia originating in basal bodies arranged in closely set rows. The cilia of the anterior region are slightly more numerous and are shorter than those of the posterior region. The ciliary rows follow the contour of the body, being nearly parallel on the ventral surface. On the dorsal surface the rows of cilia converge at the cytostome somewhat in the manner of a whorl (Fig. 1).

The cytostome is small and situated in the posterior fourth of the body on the dorsal side, about midway between the lateral edges. The two edges of the cytostome are supplied with longer cilia that beat into the opening (Fig. 1). These cilia are separate and do not form a membrane. There is no deep peristomal groove opening into the cytostome as is found in *C. mytili* (Kidder, 1933*a*), but the right end of the cytostome is pointed and continues into a narrow furrow (Fig. 1, *per. f.*). This furrow proceeds to the right lateral edge in an anterior direction, spirals around the edge to the ventral side and appears to be continuous

with the thin anterior field (Fig. 2, *ant. f.*) The anterior field ends on the ventral surface near the left lateral edge not far from the anterior end. This corresponds in position to the anterior fiber of *C. mytili* (Kidder, 1933*a*) and to the anterior field of *Ancistruma* (Kidder, 1933*b*) as the ciliary rows originate along this line.

The body is covered with a rather thick, clear pellicle that is semi-rigid. No body contraction was observed although the ciliate will flex somewhat in its creeping movements. The clear ectoplasm contains

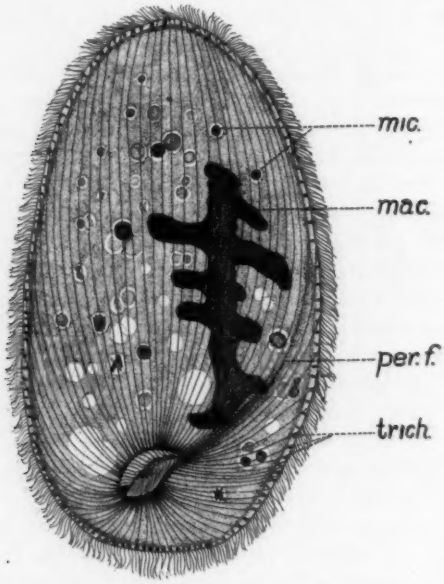

FIG. 1. *Conchophthirius caryoclada.* Dorsal view. Bouin fixation; Heidenhain's hæmatoxylin stain. × 400. Abbreviations: *mac.*, branched macronucleus; *mic.*, micronuclei; *per. f.*, peristomal furrow; *trich.*, trichocysts.

many rodlike bodies which appear to be trichocysts (Fig. 1, *trich.*). However, I have never been able to cause them to explode.

The endoplasm is finely granular and clear. Numerous food vacuoles appear in the posterior and middle region. These vacuoles are filled, for the most part, with algae taken from the water of the mantle cavity of the host. I found no host cells in these vacuoles and therefore I am inclined to regard the ciliate as a harmless commensal and not as a parasite.

Many clear vacuoles appear in the posterior region. Whether or

not these are contractile vacuoles cannot be stated at this time as no contraction was observed.

The macronucleus is located in about the mid-region of the body and slightly toward the right side (Fig. 1, *mac.*). It is very branched, the branches extending laterally from the central stem. The pattern of branching varies greatly in different individuals. The macronucleus is single, all of the branches being enclosed in a continuous membrane. The chromatin is densely granular and evenly distributed.

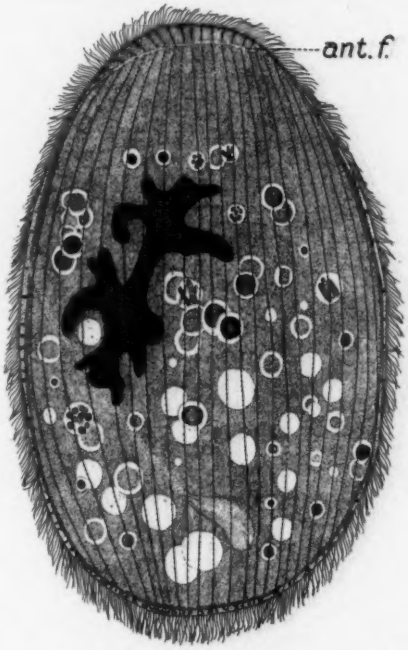

FIG. 2. *Conchophthirius caryoclada.* Ventral view. The thin anterior field, *ant. f.*, is seen at the anterior end of the body. The peristomal furrow and the cytostome are visible through the body. The chromatin of the macronucleus appears to be coarsely granular. Bouin fixation; Delafield's hæmatoxylin stain. × 400.

One or two micronuclei (usually two) are found in the anterior endoplasm near the macronucleus. They appear as dense spheres of chromatin surrounded by a clear area (Fig. 1, *mic.*). In a few forms no micronuclei could be found but this was probably due to the obscuring effect of the profusion of food inclusions.

The dorso-ventral flattening seen in the case of all members of the

genus *Conchophthirius* is carried to the extreme in *C. caryoclada*. Indeed, so flat is this ciliate that its adhesive powers make it exceedingly difficult to pick up from the bottom of the glass container. The migration of the mouth has progressed much farther than in *C. mytili* so that it has come to lie on the posterior dorsal surface. This position appears to be exceedingly advantageous, as very little if any food material could be taken into a ventrally located mouth, so closely is that surface applied to the substrate.

Conchophthirius caryoclada is unique among the members of the genus in possessing a ramifying macronucleus. It is similar to those of *Rhizocaryum concavum* (Caullery and Mesnil, 1907) and *Mrazekiella nucleoramiformis* (Kijenskij, 1925). These ciliates are astomatous endoparasites. I know of no other stomatous holotrich in which the macronucleus shows this form of branching.

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EFFECTS OF TEMPERATURE ON PRODUCTION OF MOSAICS IN HABROBRACON

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It has been shown that male mosaics of *Habrobracon juglandis* (Ashmead) arise as a result of egg binuclearity, two oötids with different genes taking part in development (Whiting, 1924). Gynandromorphs also arise in a similar way except that one of the oötids is fertilized (Whiting and Stancati, 1931).

Ordinarily, mosaics occur infrequently in *Habrobracon*. While making certain linkage tests, the author found that crosses involving shot vein, sv (wings), stock females, yielded an unusually large number of mosaic males. Subsequently, this stock was used in an experiment showing that mosaicism could be increased by X-radiation (Greb, 1933).

Previous work on mosaics of other Hymenoptera has been mainly descriptive. G. A. Rösch (1926 and 1928) reported temperature experiments with the honeybee and suggested that eggs exposed to low temperatures produce an increased number of gynandromorphs. Bee breeders in general seem to agree that a lowering of the temperature in a hive will result in the appearance of a greater number of individuals mosaic for sex.

The present work was undertaken to determine whether different temperatures might not have an effect on the ratio of mosaic production in *Habrobracon*, and if so, what temperature would produce the greatest number of these individuals.

Shot vein stock was derived by mutation from Minnesota yellow, My (base of antennæ), stock during the summer of 1930 and has been kept as a pure line since that time. Since this stock gave a higher ratio of mosaics than is usually obtained, it was utilized in these experiments. Shot vein females were mated to ivory, o^{i} (eyes), stumpy, st (legs), males. If, in an F_1 female, a binucleate egg occurred in which the two oötids carried different genes affecting eyes, wings, or legs, individuals from such eggs could be recognized. About half of the F_1 females from each fraternity were treated experimentally; the remainder were kept as controls. The latter were always reared at the usual breeding temperature, 30° C.

The method of procedure for the temperature experiments may be briefly summarized as follows:

Experiment A

The experimental F_1 females were subjected to a temperature of 5 to 10° C. in an electrical refrigerator for one hour. After treatment they were kept at room temperature for about an hour to allow them to recover. They were then given caterpillars and kept at 30° C. until time of transfer—four days. At this time the females were transferred into new vials, cooled as before, given time to recover and

TABLE I

Effects of Various Temperatures on Ratio of Mosaics

Experiment		♀♀ Set	Progeny							
			♀♀	♀♂	Males					
					Regular	♂♂	Per cent	♂♂	Differences	Mutations
A	Treated 5°–10° C.	78	895	2	1676	8	.4755±.1683		.0888±.2289	0
	Controls 30° C.	67	981	2	1543	6	.3885±.1583			1
B	Treated −14°–10° C.	147	1892	5	2526	9	.3563±.0799		.0336±.1106	0
	Controls 30° C.	115	1779	4	2499	8	.3227±.0765			0
C	Treated 18°–20° C.	192	691	0	1762	0	.0000±.0000		.2675±.0831	0
	Controls 30° C.	118	1167	2	2242	6	.2675±.1090			1
D	Treated 35°–37° C.	107	1171	5	3761	38	1.0137±.1101		.7356±.1236	1
	Controls 30° C.	95	1562	3	3989	11	.2757±.0560			2
E.	Treated 35°–37° C.	144	0	0	4900	37	.7636		——	0

The symbol ♂♂ has been suggested to designate male mosaicism and the symbol ♀♀ to designate female mosaicism; ♀♂ = gynandromorph.

then set with caterpillars for another four days. This procedure was continued for four consecutive treatments. Offspring were reared at 30° C. Results are given in Table I.

Experiment B

The treatment in Experiment B was the same as above except that the test females were subjected to — 14 to — 10° C., likewise in an electric refrigerator. This was the extreme low temperature obtainable in the ice cube chamber. The exposure was continued for one hour

at times of first two treatments and for thirty minutes at last two. It had been found that a third one-hour exposure to this low temperature was too severe, killing many of the preliminary test females. Results are shown in Table I.

Experiment C

Experimental F_1 females were given caterpillars and placed in an incubator in which a temperature of 18 to 20° C. was maintained. This temperature was kept constant by placing an incubator with an electric bulb and an automatic thermostat in an electric refrigerator. Transfers were made only about every twelve days since all the activities were slowed down by the low temperature and the young larvæ did not appear until about the twelfth day. At this time the females were transferred to new vials with fresh caterpillars and returned to the low temperature incubator. The developing larvæ were reared at the usual breeding temperature (30° C.). It was possible to make, on the average, only three transfers before the females died. Consequently, more females had to be kept at this temperature in order to obtain good numbers. Results are recorded in Table I.

Experiment D

A high temperature experiment was made with conditions similar to the last experiment except that the test females were kept in an incubator in which a temperature of 35 to 37° C. was maintained. Every fourth day the females were transferred to new vials with fresh caterpillars and returned to the high temperature incubator, while the vials of larvæ were set at 30° C. Results are summarized in Table I.

Discussion

Mosaicism

Data presented in Table I (Experiments *A* and *B*) show that short time treatments with very low temperatures do not appreciably change the rate of production of male mosaics or of gynandromorphs in *Habrobracon*. In case treatments were effective, we would expect both types of mosaics to vary in the same direction, since they come from binucleate eggs.

G. A. Rösch (1926 and 1928) reports results of short time cold treatments on newly laid eggs of the honeybee. By this means he obtained a number of gynandromorphs. However, he was not primarily interested in ratios, and consequently he gives no data regarding controls. Numbers of gynandromorphs vary irregularly with duration

and intensity of treatment, making it impossible to derive any definite conclusions regarding effects of temperature.

Habrobracon females kept always at a low temperature of 18 to 20° C. produced no male mosaics or gynandromorphs. It was thought that constant cold might slow down maturation movement within the egg in such a way that development would more frequently start from a binucleate egg. However, this certainly did not occur. There was no evidence of development from binucleate eggs, while sister females kept at 30° C. produced usual percentages of mosaic males and gynandromorphs.

A higher temperature (35 to 37° C.) than standard, approaching the upper survival limit for *Habrobracon*, did increase the rate of production of binucleate eggs to nearly four times that found among control males (see Table I, Experiment *D*, for ratios and differences). Calculations also show that the percentage of gynandromorphs among individuals from fertilized eggs is greatest at this temperature, the ratio being significantly different from the same ratio in the control group (percentage of gynandromorphs among individuals from fertilized eggs: treated, $.4269 \pm .1428$; controls, $.1914 \pm .0745$; difference, $.3355 \pm .1218$). Not a slowing down but a speeding up of maturation processes is required to obtain a large number of binucleate eggs in *Habrobracon*.

Recently another experiment was run at the same high temperature (Experiment *E*, Table I). No normal temperature control was run since the experiment was not planned to deal with ratios. However, results are consistent in showing that high temperature increases the number of individuals of binucleate egg origin. The ratio of mosaics is lower than that in Experiment *D*, probably because a new factor, honey body color, had been introduced into the ivory stumpy males.

Anna R. Whiting and Carey H. Bostian (1931) obtained a ratio of one mosaic among 1457 males (total count 6 among 8741) from diheterozygous mothers. In the experiments discussed here ratios of mosaics vary from approximately one in 260 males (Experiment *A*) to one in 370 males (Experiment *C*) in the control groups, which may be taken to indicate the range to be expected under normal conditions from the particular cross used. Temperature treatment broadens this range. Constant cold reduces the number of mosaics to zero while high temperature causes one out of about every 100 eggs to develop into a mosaic.

Percentage of gynandromorphs, as is expected, varies in the same direction as percentage of male mosaics. Among the controls, this range is one gynandromorph in 585 (Experiment *C*) fertilized eggs to one in 492 (Experiment *A*). Females exposed to constant cold pro-

duced no gynandromorphs among 691 offspring from fertilized eggs while those exposed to high temperature produced about one gynandromorph in every 235 fertilized eggs.

A comparison of the above ratios shows that there are always recorded proportionally fewer gynandromorphs than mosaic males. Both come from binucleate eggs but criteria for distinguishing the two are different. Since sex differences involve most regions of the body—antennæ, ocelli, wing size, abdominal sclerites and genitalia, as well as general body pigmentation, a gynandromorph would be more easily recognized than a male mosaic for some trait affecting a limited region such as eyes or wings. Since polyspermy occurs in eggs of Hymenoptera, fertilization of both nuclei of a binucleate egg would presumably lead to the production of a female rather than a gynandromorph.

Goldschmidt (1931), in his work on *Bombyx mori* L., obtained a large number of individuals (mosaic males, mosaic females, and gynandromorphs) which must have come from binucleate eggs in which both nuclei had been fertilized. Sex determination is different in *Bombyx* than in *Habrobracon* and both nuclei must be fertilized in order that an individual can be developed.

An effort has been made to obtain mosaic females in *Habrobracon*. Females heterozygous for shot vein, ivory, honey, and stumpy were mated to shot vein, ivory, honey, and stumpy males. It was hoped that the two oötids of a binucleate egg, differing by at least one factor, would be fertilized by recessive sperm and that a female mosaic would appear. The 69 females set produced: 692 females, 2 gynandromorphs, 880 males, and 2 mosaic males. No female mosaics were observed. However, the numbers are small and further tests must be made to determine whether polyspermy may not occur in binucleate eggs in *Habrobracon*. Two gynandromorphs with patroclinous male parts have been interpreted as being derived from binucleate polyspermic eggs (Whiting, 1931).

An individual was recently found by Magnhild Torvik Greb which can best be explained by assuming polyspermy in a binucleate egg. The individual is a female having left antenna aciform, right antenna type, left ocellar patch different in pigment from right and left gonapophysis shorter than right. Aciform, ac, causes reduction of diameter and often fusion of segments of approximately one-third of the antennæ at the distal end. It also reduces the size of the sensory gonapophyses, usually causing asymmetry. In this female, the left antenna was modified aciform, only the most distal segments being affected and these but very slightly—probably due to the fact that type tissue was also present. The genitalia were asymmetrical, the shortened gonapophysis being on the side with the aciform antenna. However, it cannot be definitely stated

that the genital region is mosaic. This mosaic female appeared in a fraternity from a mother heterozygous for aciform which had been mated to an aciform male. Its mosaicism may be explained by assuming polyspermy and that the two oötids of a post-reduced binucleate egg (one carrying aciform, the other the normal allelomorph) were fertilized by sperm carrying the recessive aciform. Thus we could obtain a female, part aciform and part type.

Sex Ratios

X-radiation of mated females has been shown to affect sex ratio of their offspring as well as their fecundity (Whiting, 1929; Bostian, 1931). X-radiation increases rate of appearance of mosaics. It seemed possible that high temperature, which also increases the ratio of mosaics, might similarly affect sex ratio and fecundity.

TABLE II

Sex Ratio in Bisexual Fraternities

	Experiment	♀♀ Set	Progeny					
			♂♂	♂♀	♀♀	Per cent ♀♀	Sons per mother	Total per mother
A	Treated 5°–10° C.	61	1100	2	895	44.9	18	33
	Controls 30° C.	53	983	2	981	50.0	19	37
B	Treated −14°–10° C.	140	2469	6	1892	43.5	17	30
	Controls 30° C.	107	2226	4	1779	44.6	19	37
C	Treated 18°–20° C.	111	1101	0	691	38.6	10	16
	Controls 30° C.	78	1663	2	1167	41.2	21	36
D	Treated 35°–37° C.	76	1966	5	1171	37.3	26	41
	Controls 30° C.	65	2187	3	1562	41.6	34	58

A summary of offspring in bisexual fraternities is given in Table II. This summary brings out the fact that in all temperature experiments percentage of females is slightly lower among the offspring from treated than among the offspring from control females.

Fecundity

Fecundity of females, however, is more dependent upon temperature than is sex ratio. Again treated females always produced fewer offspring per mother than control females but all treatments did not have

quite the same effect. The fecundity of the treated females in Experiment *A* was slightly decreased as compared with that of its control females. In Experiment *B*, there was a marked decrease in the number of offspring from treated as compared with offspring from controls. The very high temperature, Experiment *D*, reduced the fecundity by a little less than a third while the constant cold temperature decreased it by more than one-half.

Constant cold and extreme heat both had very significant effects on fecundity though they apparently acted in different ways. Cold acted by slowing down all the activities of the adult wasps thereby decreasing the rate of egg production. Cold also kept many of the eggs that were laid from hatching and killed many of the young larval wasps. Heat treatment apparently had little or no effect on rate of egg production. It affected the young, the consequences being especially noted in the pupal stage. There were many naked pupæ and many dead in the cocoons.

Anomalous Types

During the course of these experiments five mutant individuals were observed—each in a different fraternity. Four carried recurrent mutations to orange—a not infrequent mutation—from mothers heterozygous for ivory, Oo^{i}, an allelomorph of orange (Whiting, 1932, p. 297). These occurred in the controls. The fifth was at first mistaken for a gynandromorph with a female head and a male abdomen. However, the individual mated with a female and the trait, called gynoid, gy, was inherited as though due to a simple recessive factor. Gynoid males have female-like antennæ and abdominal sclerites. Homozygous gynoid females are indistinguishable from type. The first gynoid male, the mutant, appeared in the first of three vials of offspring from a heat-treated female (Experiment *D*, Table I).

Numerous other anomalous individuals were noted. Wasps with one large medial eye, "cyclops," were rather frequent among offspring from mothers treated with high temperature.

Summary and Conclusions

1. Short time exposure of heterozygous females to extreme cold did not significantly affect rate of production of male mosaics and gynandromorphs.

2. When such females were placed at a constant low temperature no mosaic offspring appeared.

3. Females kept at 35 to 37° C. produced a significantly greater number of such individuals than appeared in any other case.

4. Temperature treatment of mated females slightly affects the sex ratio of offspring, the most significant decrease in female ratio being observed at constant low or high temperatures.

5. Temperature treatment affected fecundity although short time exposure to cold had only a slight effect. Constant high temperature decreased fecundity by a little less than a third and constant low temperature lowered it to less than half that of the control wasps.

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THE DIFFERENTIAL EFFECT OF ENVIRONMENTAL FACTORS UPON MICROBRACON HEBETOR SAY (HYMENOPTERA: BRACONIDÆ) AND ITS HOST EPHESTIA KÜHNIELLA ZELLER (LEPIDOPTERA: PYRALIDÆ). I

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Introduction and Literature

A change in environment either physical, as temperature, light, or humidity, or biological, as the introduction of a new parasite, a predator, or a disease, may upset the equilibrium established between host and parasite. Certain conditions may favor the host; others, the parasite. Escherich and Miyajima (1911, 1912) described an interesting relation between the polyhedral disease in the nun moth, *Porthetria monacha* Linn., and weather changes. The latent form of the disease developed into the chronic, fatal form when nun moth larvae were exposed either to bright sunlight or to decrease in temperature. Field studies showed that outbreaks of the disease were correlated with sudden changes in weather. Růžička (1924, 1925) found that there was a periodicity in the outbreaks of the nun moth and that these outbreaks were associated with sun spot cycles. Weather cycles affected the parasites more than the hosts. If the spring temperature is high and the humidity low, the host is favored; if the spring temperature is low and the humidity high, the parasites. Both Tachinidae and fungi, parasitic on *Porthetria monacha* Linn., are favored by the same factors of high humidity and low temperature.

One of the most carefully studied cases of the differential action of environment on host and parasite is that of the "green bug" or grain aphis, *Toxoptera graminum* Rond., and its chief parasite, *Aphidius testaceipes* Cress. Webster and Phillips (1912) approached the problem of balance between the "green bug" and its parasite by determining the threshold for activity of each. The host was active and able to oviposit at a temperature lower than that required for the parasite. Warm weather was more favorable to the parasite than to the "green bug" itself. Headlee (1914) again attacked the problem of balance between host and parasite with the aid of special apparatus for controlling humidity and temperature.

From the data of Hunter (1909) on the number of aphids infested

by a single parasite, from the results of Glenn (1909) on developmental threshold, and from the data of Headlee (1914) on the effect of temperature and humidity on host and parasite, Shelford (1926) came to the conclusion that it was impossible theoretically for the parasite to overtake the host. Shelford's method of determining a threshold of development was quite similar to that of Sanderson (1910) and Krogh (1914), but he used a theoretical α in place of the developmental zero of these authors. He also summed up developmental units instead of temperature, and concluded that since the parasite required more developmental units than the host, it could not exterminate the host.

The differential effect of environment on host and parasite has been worked out for the beet fly, *Pegomyia hyoscini* Panz., and its parasites; the gall midge, *Rhabdophaga heterobia* H. Lw., and its parasites; and the hawk moth pupa, *Protoparce quinquemaculatus* Haw., and its parasites.

According to the researches of Bremer (1928), and Blunk, Bremer, and Kaufmann (1928), cold weather retards the development of the parasites, *Opius fulvicollis* Thoms. and *Phygadeuon pegomyiae*, of the beet fly, *Pegomyia hyoscini* Panz., more than it does the host. Thus during a cool summer the beet fly gains on its parasites. In warm weather the parasites gain on it.

Barnes (1930) found that the parasites of the gall midge, *Rhabdophaga heterobia* H. Lw., were less cold-hardy than the midge itself. The percentage of survival of both *Dasyneura alopecuri* Reuter and *D. pyri* Bouché after exposure to low temperature was less than their host. This conclusion was based largely upon material collected in the field.

Hefley (1928) showed that as the relative humidity increased, the viability of the hawk moth pupa, *Protoparce quinquemaculatus* Haw., decreased. The viability of the parasite, *Winthemia quadripustulata* Fab., varied directly with increasing relative humidity. Above 73.4 per cent relative humidity emergence of the parasite was practically 100 per cent. In other words, a low relative humidity was favorable to the host; a high one, to the parasite.

Host-parasite balance may be affected not only by a physical factor of the environment, but also by a biotic factor. The effect of competition between parasites may be considered as the effect of a change of biotic environmental resistance on the balance between a single host and parasite. Important as is this problem to biological control work there is very little data upon it. Pemberton and Willard (1918) found that the total parasitic infestation of the fruit fly,

Ceratitis capitata Wied., decreased when to the very efficient parasite, *Opius humulis* Silves., was added a less efficient parasite, *Diachasma tryoni* Cam. Larvæ of this parasite killed the larvæ of *Opius humilis* Silves. with their sharp mandibles. The killing of *Opius* by *Diachasma* was undesirable since *Opius* survived the winter better than *Diachasma*.

Literature on Host-Parasite Balance of Ephestia kühniella Zeller and Microbracon hebetor Say

Efforts to evaluate *Microbracon* as a control measure for its host, *Ephestia*, have to a certain degree been attempts to determine the balance between this particular host and its parasite.

Buchwald and Berliner (1910) considered that *Microbracon hebetor* Say (*Habrobracon hebetor* Say) was a valuable aid in control of the Mediterranean flour moth. Back (1920) mentioned *Microbracon hebetor* Say and *Omorga frumentaria* Rond. as accessory controls for *Ephestia*, but thought that heat treatment or fumigation must be relied upon for effective combat.

Hase (1922, 1925) made an attempt to evaluate the parasitic efficiency of *Microbracon hebetor* Say (*Habrobracon brevicornis* Wesmael.) in controlling *Ephestia kühniella*. He let loose 100 *M. hebetor* adults and 500 *E. kühniella* larvæ in a space with a cubic content of 9,264 cubic meters with a surface of 387,348 square meters. He recovered 129 host larvæ and of these 70 were parasitized. Later he conducted similar experiments in which he found a parasitism of 50 to 60 per cent. From the concentration of *Microbracon hebetor* necessary to parasitize 50 per cent of the flour moth caterpillars he calculated that 20,000 female *M. hebetor* per 1,000 cubic meters would be needed for complete control. Hase did not reckon on any cumulative effects of several generations of parasites, but only on one. However, Muir (1913) has shown that a parasite may be overtaking a host when the host is apparently gaining.

Methods and Materials Used in the Present Experiments

Two genetic strains of the Mediterranean flour moth, one of which developed much more rapidly than the other, were used in these experiments. Pure lines of the parasite, *Microbracon hebetor* Say, were not kept, but there was little variation between the reactions of different individuals to temperature. The females were almost pure yellow at 30° C. and almost black at 16.5° C. The stock was remarkably free from abnormal individuals. No twisted wings, white eyes, or stumpy antennæ were observed. Undersized adults were produced

only when too many parasite larvæ fed on one host caterpillar, or when parasites fed on pink host larvæ.

Temperature studies on *Microbracon hebetor* Say were begun at the Biologische Reichsanstalt in Berlin-Dahlem and continued at the Marine Biological Laboratory at Woods Hole, Massachusetts, and at the University of Pennsylvania. The temperature equipment here described is that at the Anstalt in Dahlem. With the exception of the series incubators, the apparatus at Woods Hole and at the University of Pennsylvania was similar to that used at Dahlem.

The temperature control apparatus at Dahlem consisted of incubators in series, one at low temperature, the other at high, and also of individual incubators. There was no circulating air in them. For the insects in question the lack of circulating air is not a serious fault, but it would be for insects eating green food. The low temperature series of incubators was kept in a basement which itself was at low temperature. The compartments in the series incubator were kept at 16.5° C., 11.5° C., 9° C., 8° C., 5° C., 3.5° C., 2.2° C., and .9° C. The average deviation from the temperatures in question was ± .5° C. and the greatest deviation ± 1.0° C. Temperatures were read twice daily. The high temperature series was less accurate than the low. Gas was used as the source of heat. Temperatures of 38° C., 36.5° C., 32° C., 30° C., 28° C., 27° C., 25.4° C., 23.5° C., and 19° C. were maintained in the high series. The first three compartments on the high side and the first two on the low did not vary from the mean more than ± 1° C. The others could be used only for rough comparative studies to block out limits of duration of different stages. These limits could then be determined more accurately with individual incubators. In one series of experiments the compartment at 36.5° C. fell to 34° C., at which temperature it remained constant. Since the temperature remained constant at 34° C., opportunity was given to rear two generations of *Microbracon hebetor* and one generation of *Ephestia kühniella*. In all incubators, but in the series ones especially, it is important that the thermometer used for reading the temperature be at the same level as that at which insects are kept. The thermometers at the tops of the series compartments could not be used as fair indicators of the temperatures inside.

Individual incubators kept at 10° C., 16° C., 25° C., 27° C., and 30° C. were used. Two rooms, one kept at 18°–19° C., and the other at 15° C., were also available. Since it is much more difficult to maintain a constant temperature lower than the external environment than to maintain a temperature higher, the best results for constant low temperatures were obtained by placing an incubator in a room lower than

the temperature desired. A room at 4° C. was used for the 10° C. and 16° C. incubators.

Host larvæ were reared in Petri dishes, in cylindrical jars, and in cardboard boxes. They were fed on rolled oats. In experiments to determine host-parasite balance, 10 adult parasites were placed with 100 host larvæ, eggs, or pupæ.

Thompson (1922) proposed a formula, later corrected by Chapman (1925), which could be used to calculate the number of generations required to overtake a host. A simple case was assumed, in which the parasite laid only one egg on each host attacked. Environmental factors were supposed to act equally favorably on both host and parasite.

The formula will be developed as follows:

If n = the original population of the host,
l = the sex ratio of the host,
h = the number of young produced by each female host,
Then, $lh = w$, the reproductive power of the host (reproductive potential).
If p = the original population of the parasite,
f = the sex ratio of the parasite,
s = the number of young produced by each female parasite,
Then, $fs = z$, the net reproductive power of the parasite (reproductive potential).

Then, nw = the number of hosts after the first generation,
pz = number of parasites after the first generation,
$nw - pz$ = number of surviving hosts after the first generation.

k = number of parasite generations to equal host population.

The number of generations, k, required for the number of parasites to equal the number of hosts is found when

$$(nw^k \cdots) - pz^k = 0,$$

or the number of parasites is equal to that of the host. k may be found by using the mathematical transformation

$$\theta = \frac{w}{z}, \qquad \beta = \frac{zp}{n(z - w) - zp} \qquad \text{and} \qquad k = \frac{\text{Log } \theta}{\text{Log } \beta}.$$

If w is greater than z or in other words, if the reproductive power of the host is greater than that of the parasite, p can never equal h, and there is no theoretical possibility that the parasite can exterminate the host.

TABLE I

Development of Ephestia kühniella

Temperature	Time required for fast strain	Time required for slow strain	Number of generations per annum of fast strain	Number of generations per annum of slow strain
° C.	*days*	*days*		
32	35– 37	62– 65	10.4–	5.8
30	41– 43	66– 68	8.9–	5.5
27	43– 45	69– 72	8.5	5.3
25	50– 53	79– 81	7.3	4.6–
20	63– 65	99–103	5.8	3.7–
15	85– 90	135–157	4.3	2.6–
10	140–175	205–243	2.6	1.08
8	cannot develop	250–270	—	1.04

If $w = z$, or in other words, the reproductive powers of the host and parasite are equal, then

$$k = \frac{h}{p},$$

or the number of generations required by the parasite to equal the numbers of the host is equal to the quotient of the original number of hosts, divided by the original number of parasites.

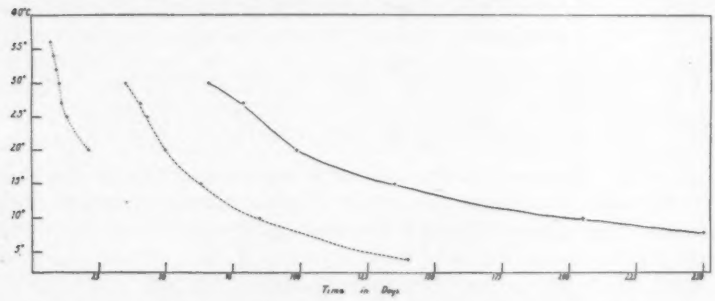


FIG. 1. Duration of development of host and parasite from the time the egg is laid until the emergence of the adult, plotted against temperature.

Dotted line, parasite (*Microbracon hebetor*). Broken line, fast strain of host (*Ephestia kühniella*). Solid line, slow strain of host (*Ephestia kühniella*).

However, *Microbracon* may lay as many as 22 eggs on a single host. The number of eggs laid on a caterpillar stung by the host varies from 0–22, with a mean value of 10. If a parasite lays 10 eggs on one host, it does not affect the host population as much as if it laid one egg on each of 10 hosts. The number of generations required

TABLE II

Development and Length of Life of Microbracon hebetor

Temperature	Time required for development	Number of generations per annum	Median life length of female
° C.	*days*		*days*
36	7	52–	3
34	8	45.6	6
32	9	40.5	9
30	10–11	36.5	13
27	11–12	33.2	20
25	13–14	28.7	23
20	19–20	19.2	28
16.5	—	—	55
10	—	—	90

for the parasite to overtake the host when the parasite lays more than one egg per host may be expressed by the following equation,

$$k = \frac{\log \theta}{\log \beta} = \frac{\log \dfrac{w}{\dfrac{z}{m}}}{\log \dfrac{\dfrac{zp}{m}}{n\left(\dfrac{z}{m} - w\right) - \dfrac{zp}{m}}}$$

with the additional symbol m, which represents the number of eggs laid per host by a parasite. For the expression z/m, a new symbol, ϵ, may be used to designate parasitic efficiency. Then the equation reads,

$$k = \frac{\log \dfrac{w}{\epsilon}}{\log \dfrac{\epsilon p}{n(\epsilon - w) - \epsilon p}}.$$

In order to determine the effect of temperature upon host-parasite balance, both strains of the host and the one strain of parasite were reared separately. Then host and parasite were put together.

RESULTS OBTAINED

Effect of Temperature upon Life Cycles of Host and Parasite

The slow strain of host can survive and complete its developmental cycle at temperatures from 34.4° C. to 8° C. inclusive. The larva of the slow strain can live at 36° C., but cannot pupate. The fast strain of host cannot complete its developmental cycle at temperatures

below 10° C. The time required from deposition of the egg to emergence of the imago is shown in Table I. As will be seen by the table, the potential number of generations per annum of the two strains differs widely at each temperature. Although the durations of the life cycles of the slow and of the fast strain differ markedly, yet

TABLE III

Reproductive Potential of Ephestia kühniella

Temperature	Eggs laid per female	Sex ratio	* Fertility of fertilized females	Reproductive potential
° C.				
32	181	54	.70	68.618
30	204	54	.70	77.108
27	225	54	.70	85.05
25	200	54	.70	75.60
20	177	54	.65	62.127
15	156	54	.50	42.12
10	67	54	.10	3.618
8	55	54	.10	2.97

* Fertility expressed as percentage of females laying eggs.

TABLE IV

Reproductive Potential of Microbracon hebetor

Temperature	Eggs laid	Sex ratio	Reproductive potential	Parasitic efficiency
° C.				
36	90	.75	67.50	6.75
34	105	.75	78.75	7.875
32	111	.75	83.25	8.325
30	114	.75	87.50	8.75
27	120	.75	90	9
25	113	.75	85	8.5
20	87	.75	65.25	6.52
15	56	.50	28	2.8
10	—	—	—	—

the effect of temperature on the rate of development of the two strains is approximately the same. The similarity of the curves of the two strains made when duration of development is plotted against temperature is shown in Fig. 1.

The parasite, *Microbracon*, can live and complete its life cycle at temperatures from 36° C. to 14.5° C. The adult could survive for

long periods at lower temperatures, but could not lay eggs. Length of time from egg to emergence of adult, and the median life length of the adult female as it varies with temperature, are shown in Table II. The curve produced when temperature is plotted against duration of development is shown in Fig. 1.

Not only is the duration of development affected by temperature, but the reproductive ability also. The biotic potential at any given

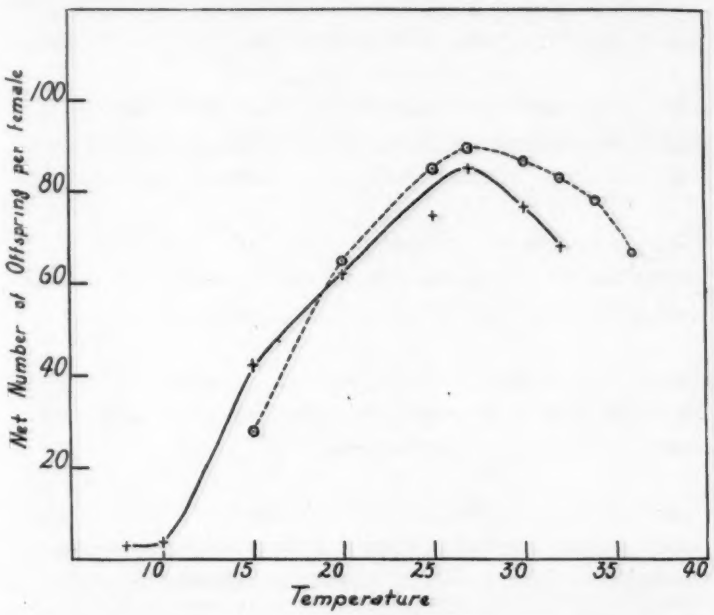
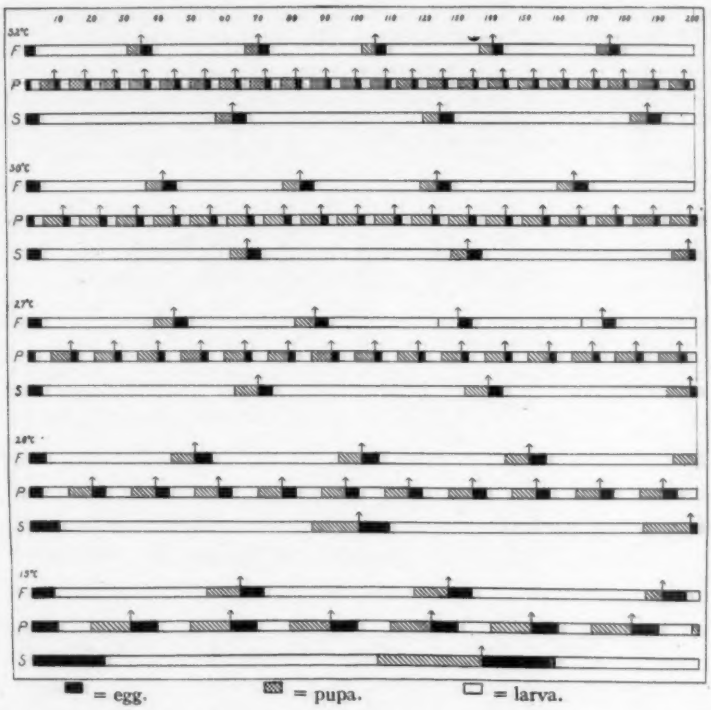


FIG. 2. Reproductive potential of host and parasite at the different temperatures studied. Solid line, *Ephestia kühniella* (host). Broken line, *Microbracon hebetor* (parasite).

temperature may be calculated by multiplying the average number of eggs laid per female, by the decimal representing the percentage of the population which is female, and this product by the percentage of females which lay eggs. Fertility is used in the statistical rather than in the physiological sense. The biotic potential of *Ephestia*, the host, is shown in Table III; that of *Microbracon*, the parasite, in Table IV. The maximum biotic potential of both host and parasite is reached at 27° C., as shown in Fig. 2.

As seen from Table V, or better from Fig. 3, the life cycle of the parasite is characterized by a comparatively short larval stage and a relatively long pupal stage. The host has a relatively long feeding stage and rather short resting stages, namely, the pupa and the egg.



F = fast strain of host. S = slow strain of host. P = parasite. † = emergence.

FIG. 3. Length of egg, larval, and pupal stages, and the time of emergence of two strains of host (*Ephestia kühniella*) and one of parasite (*Microbracon hebetor*).

Since the life cycles of the host and the parasite are not synchronous, the possibility of the parasite catching up with the host had to be determined under two conditions, (1) when the parasite emerged during a period of the host on which it could feed, and (2) when the parasite emerged during a period of the host on which it could not feed. The parasite can feed on the larval stage only of the host.

Host-parasite Balance if the Parasite is Set on the Larval Stage of the Host

The ability of the parasite to control the host depends upon its relative reproductive rate and also upon its relative life length in comparison with those of the host. The initial number of hosts in the experiments to be described was 100; the initial number of parasites, 10. Results of some of the experiments in host-parasite balance are shown in Table VI.

TABLE V

Time Required for Development

Temperature	Ephestia kühniella								Microbracon hebetor			
	Slow strain				Fast strain							
	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total	Egg	Larva	Pupa	Total
° C.	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
36	—	—	—	—	—	—	—	—	1	3	3	7
34	—	—	—	—	—	—	—	—	1	3	4	8
32	4	53	5	62	3	28	4	35	1.5	3	4.5	9
30	4	57	5	66	4	32	5	41	2	3	5	10
27	4	58	7	69	4	33	6	43	2	3	6	11
25	5	64	10	79	5	38	7	50	2	5	6	13
20	9	76	14	99	7	46	10	63	4	8	7	19
15	22	82	31	135	18	53	14	85	8	10	12	30
10	34	122	49	205	27	75	38	140	—	—	—	—
8	43	143	64	250	—	—	—	—	—	—	—	—

At temperatures from 32° C. to 20° C. inclusive the parasite was able to overtake the host. As shown in Table VI, at the close of the first generation the numbers of the host were not always appreciably diminished. However, by the end of the second generation the host was annihilated. The possibility of the parasite overtaking the host is not only a theoretical possibility, but also a practical fact that has to be taken into consideration when rearing hosts and parasites. A large extra supply of host larvæ must be kept on hand in order to keep the parasite culture going, since the parasites in killing off their hosts also destroy their own food supply.

Under the conditions of the experiment the parasite required 18 days to overtake the host at 32° C., 20 days at 30° C., 22 days at 27° C., 26 days at 25° C., and 39 days at 20° C.

At 15° C. the parasite was able sometimes to overtake the host, sometimes not. The possibility depended upon whether or not the

parasite was able to complete two generations on the host larvæ. There was more variation both in generation length of the parasite and in the length of the host larval period at extreme temperatures than at medial temperatures. If the slow strain of host larva required

TABLE VI

Host-parasite Balance at the Different Temperatures Used

Temperature	Larval period of host		Length of parasite life cycle	Initial number of parasites	Initial number of hosts	Hosts killed by parasite, 1st generation	Hosts remaining	Parasite potential by second generation	Hosts remaining
	Slow strain	Fast strain							
° C.	*days*	*days*	*days*						
32	53	28	9	10	100	83.20	17.80	6952	—
30	57	32	10	10	100	87.50	12.50	76542.5	—
27	58	33	11	10	100	90	10	81000	—
25	64	38	13	10	100	85	15	72250	—
20	76	46	19	10	100	76	24	57760	—
15	82–90	53–62	30–40	10	100	28	72	*784.00	—

* If host larva in fast strain requires only minimum time the parasite can never catch up with it.

TABLE VII

Comparative Life Length of Adult Parasite and Non-feeding Stages of the Host

Temperature	Life length of adult parasite	Duration of egg stage of host		Duration of first larval stadium		Duration of egg + pupa of host		Duration of pupa + egg + 1st instar larva of host		Duration of egg + 1st instar	
		Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain	Slow strain	Fast strain
° C.	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
32	9	4	3	8	4	9	7	17	11	12	7
30	13	4	4	8	5	9	9	17	14	12	9
27	20	4	4	9	6	11	10	20	16	13	10
25	23	5	5	11	7.5	15	12	26	19.5	16	12.5
20	28	9	7	12	9	23	17	35	26	21	16
15	53	22	18	18	12	53	32	71	44	40	36
10	90	34	27	—	—	—	—	—	—	—	—

the minimum time to develop, namely 82 days, and the parasite the minimum time, the parasite would be able to catch up with the host. If the parasite required the maximum period for development, namely 40 days, and the slow strain of host 82 days, the parasite would not be able to produce two generations on the host larva nor to overtake

the host, when allowance is made for the length of the first instar of the host larva on which the parasite cannot feed.

If the fast strain of host requires the minimum period in which to develop and the parasite also requires the minimum, the parasite can produce only one generation on the host larva, and cannot overtake the host. In order for even the most rapidly developing parasite to reproduce twice on a single generation of host larva, the larval period of the host must be at least 60 days long. Even on the slowest developing host larvæ of the fast strain, the parasites are unable to produce more than one generation, if they require more than 31 days for a life cycle. The chances that 10 parasites will be able to overtake 100 fast strain hosts when set on fast strain larvæ at the beginning of the larval period are about 1 : 16, if there is no pre-imaginal mortality among the parasites.

However, at 15° C. there is often pre-imaginal mortality among the parasites. A drop of water may condense on the dish in which the culture is kept and the parasite may drown in it. Drowning of young parasites may be set down to poor technique. Even if the dishes are kept free from water of condensation, young parasites die. At 15° C. the adult parasite is very clumsy. The female may miss the ganglion when she stings the host caterpillar, thus failing to paralyze the host larva and consequently failing to provide food for the young parasite. Eggs are often laid some distance away from the host larva. At 15° C. the female parasite may lay eggs so far away from the host larva that the parasite larvæ die of starvation before they are able to find their food. Not only is the adult clumsier, but also the young larva seems to be more poorly coördinated than at high temperature. If as many as three larvæ of the first generation of the parasite die, the parasite is unable to overtake the fast strain of host.

Below 15° C. there is no possibility of the parasite catching up with the host, since the parasite cannot complete its life cycle, although the adult can survive temperatures of 0° to + 4° C. for a period of several months. It is also of importance to realize that only the females which have been reared at low temperatures are capable of laying eggs at 14.5° C. and 15° C.

At temperatures above 32° C. the parasite has a decided advantage over the host. The maximum temperature at which the host can complete its life cycle is 34.4° C., but the parasite is able to complete its life cycle at a temperature as high as 36° C. The larval stage of the host, however, can survive 36° C., and thus provide food for the parasite.

Host-parasite Balance if the Parasite is Set upon a Host Stage on which it Cannot Feed

Since the life cycles of the host and parasite are not synchronous, parasites may emerge at a time when there is no food for them. They cannot feed on the egg, the pupa, or the first larval stadium. If adult parasites emerge at the beginning of the pupal period of the host, they must wait through this period, through the emergence and sexual maturity of the adult host, through the egg stage of the adult, and in addition through the first larval stadium. Since the adult female of *Ephestia* generally lays eggs on the day in which she emerges, the preoviposition period of the host is calculated in with the egg stage. If the parasite emerges at the beginning of the egg stage it must survive both this stage and the first larval stadium. Table III shows the median life length of the adult parasite as compared with the host stages upon which it cannot feed.

If the adult parasite is set on the slow strain of host at the beginning of the pupal stage it cannot survive at 32° C., 30° C., 25° C., 20° C., or 15° C. The parasite can survive at 27° C., at which temperature the biotic potential of both host and parasite reaches its maximum. Not only can the parasite survive, but it can also overtake the host under certain conditions. If the parasite is able to lay viable eggs on the last day of life the parasite can survive. For the strain of *Microbracon* used, the number of eggs laid each day was practically constant throughout life. Therefore, if the net number of offspring for each parasite during its entire life was 90, the number of offspring left by a parasite the last day of its life would be one-twentieth of 90, or 4.5. For 10 *Microbracon* the number would be 45, which offspring would be capable of exterminating 4.5 hosts. At the end of the first generation of parasites, the host numbers would have decreased but slightly. However, these 45 individuals produced would have their full reproductive power, and could produce 4050 offspring, capable of parasitizing 405 hosts. In the meantime the 100 original hosts would have increased their numbers to 7715, when the emerging adults laid eggs. At the end of the second generation of parasites the number of hosts would be 7310. The third generation of parasites would consist of 364,500 individuals capable of parasitizing 36,450 hosts, or several times the hosts remaining.

If the 10 original parasites produce only 10 offspring, or one per parasite, the parasites will yet be able to overcome the host by the end of the third parasite generation.

If the adults of *Microbracon hebetor* are set on the fast strain of host at the beginning of the pupal period they will be able to survive

at all temperatures from 27° C. to 15° C. inclusive, and to overtake the host at 27° C., 25° C., and 20° C., but not at 15° C. At 15° C., even when there is a stage of the host present upon which the parasite can feed, the reproductive power of the parasite is insufficient to overtake the fast strain of host. When the effective reproductive power is lowered by a period of starvation, the parasite is unable to overtake the host.

If the adult *Microbracon* are set on either strain of host at the beginning of the egg stage, the *Microbracon* are able both to survive and to overtake the host, except at 15° C.

When the survival of the host depends upon its ability to starve out the parasite, the slow strain has the advantage, since the pupal and the egg periods are longer in it. When survival of the host depends upon a rapid enough development in the larva to prevent two generations of parasites from developing, the fast strain of host has the advantage. Since the larval period of either host strain is longer than the combined duration of the other periods in the life cycle (see Fig. 3), the fast strain has the advantage of the slow one, considering the life cycle as a whole.

Humidity as Affecting Host-parasite Balance

The adults of the parasite, *Microbracon hebetor* Say, are extraordinarily resistant to low relative humidity. They can be kept over anhydrous $CaCl_2$ throughout life with no apparent harm. The eggs, spun larvæ, and pupæ are also resistant to low relative humidity. The larval stage, which is of comparatively short duration as compared with the other stages in the life cycle, is the only stage which can be injured easily by an atmosphere of low relative humidity. Although the larvæ are not as resistant to drying as are the other stages, even they are injured and may die in a saturated atmosphere. At 15° C. young larvæ frequently die if the relative humidity is not kept low by an appropriate salt. The larvæ are sensitive to relative rather than to absolute humidity, since the absolute humidity at saturation at 15° C. is less than the absolute humidity with a 50 per cent saturation at 20° C., for example.

The host, *Ephestia kühniella* Zeller, is sensitive to low relative humidity. Low humidity is associated with increased sterility in the adults and with production of large numbers of crippled and deformed individuals. Low humidity during the pupal stage especially is reflected by appearance of deformities in the adult stage. The larvæ are able to withstand but little drying. They prefer damp food to dry and can with difficulty gain foothold on sound, dry products.

Once, however, the larvæ infest a given material, they raise its moisture content and thus make the environment favorable for more of their species.

The host, *Ephestia kühniella*, cannot live and reproduce under the low relative humidities that the parasite can; the parasite cannot live in the damp atmosphere that favors the host. At intermediate humidities both can live and reproduce.

Factors Other than Physical which Influence Host-parasite Balance

The color of the host larvæ, the presence of "*Microbracon* immune" strains, and the opposite reactions of host and parasite to light also influence host-parasite balance.

There are two colors of full-grown host larvæ, a pinkish and a white or greenish. The pink larvæ are unable to support as large a population of *Microbracon* as are the white ones. In a mixed group of pink and white host larvæ the female parasite stings the white larvæ before she does the pink ones. The chemical nature of the pigment difference between the two strains of larvæ is not as yet known. Color of the larvæ is, however, an inheritable character. Complicating factors in determining the genetics of color are changes of color with advancing age of the larvæ, and the effect of certain environmental factors on color.

In a given population of host larvæ exposed to parasitic attack some may be immediately stung by the parasites. Others may be oviposited upon when the choice of host larvæ has become very limited. Finally, there exist certain host larvæ which are apparently "*Microbracon* immune." These are not stung by the parasitic wasps even if there are no other larvæ available.

The degree of *Microbracon* immunity of *Ephestia* larvæ was determined by allowing 10 *Microbracon* access to 100 *Ephestia*. At the end of the first day those *Ephestia* which were not stung were exposed to fresh *Microbracon*. If they were not stung by the second day they were exposed a third and so on, until the only larvæ remaining were those which the *Microbracon* did not attack. About one larva in 3000 appeared to be absolutely immune to the strain of *Microbracon hebetor* used in the experiments.

The *Microbracon* adults are strongly positively phototropic and the *Ephestia* larvæ negatively phototropic. The adult parasite generally flies directly toward the source of light when it emerges, even if host larvæ are present. Host larvæ avoid light.

When mixed populations of host larvæ and parasite adults were kept in cylindrical jars or Petri dishes, the parasite was able to

exterminate the host. When, however, the cultures were placed in square cardboard boxes the hosts were able to survive. They would spin up in dark corners. Once covered with a cocoon they were attacked with difficulty by the *Microbracon* adults. In the square, cornered containers the better protection of the larvæ apparently was not due alone to their light reactions, but also to their strong positive thigmotropism.

Presence of disease in the host larvæ may affect balance between the insect parasite and its host. If a host larva becomes infected with a fatal disease, the parasites upon it die with it. In the early stages of both sporozoan and bacterial infection the infected larvæ move about more slowly than do normal larvæ and are more subject to parasitic attack. The elimination of the parasites feeding on diseased larvæ may in some cases enable the host population to survive, even when it loses some of its members through disease. In general it may be said that if a parasitized larva becomes diseased, the host-parasite balance is tipped in favor of the host; if a non-parasitized larva becomes diseased the host-parasite balance is tipped in favor of the parasite. The above conclusion holds only if the disease is a fatal one. If the disease is non-fatal, it influences host-parasite balance if it makes the host larva a less nourishing food for the parasite or if it delays maturity of the host. In the former case the host would be favored; in the latter, the parasite.

Discussion

When disease is not present and "*Microbracon* immune" host larvæ do not form a significant part of the host population, the Thompson formula as modified by Chapman predicts the possibility of a given parasite population exterminating a host population. In the original use of this formula, the assumption was made that the life cycles of host and parasite were synchronous. If these cycles are not synchronous, as is the case with *Ephestia* and its parasite, *Microbracon*, results calculated from the formula agree fairly well with those obtained from experiment, if account is taken of the decreased reproductive ability of the parasite, when it emerges during a stage of the host upon which it cannot feed.

Summary

1. Two strains of the Mediterranean flour moth, one of which develops much faster than the other at a given temperature, were used in the experiments reported in this paper. Only one strain of the parasite, *Microbracon hebetor* Say, was bred.

2. If the parasite emerges during the larval stage of the host and is able to complete two generations during this stage it can exterminate the host.

3. If the parasite emerges at the beginning of the pupal period of the host it can exterminate the slow strain of host only at 27° C., but it can overtake the fast strain at temperatures from 27° C. to 20° C. inclusive. If the adult parasite emerges at the beginning of the egg stage it can exterminate either strain of host except at 15° C. or below.

4. In general high temperatures (above 32° C.) favor the host; low temperatures (15° C. and below), the parasite.

5. High relative humidity favors the host; low relative humidity, the parasite.

6. Conditions other than physical, such as disease incidence and the webbing habits of the flour moth caterpillar, also affect the host-parasite balance.

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STUDIES OF THE MITOTIC FIGURE

III. Chætopterus: Central Body Structure at Metaphase, First Cleavage, After Using Diluted Fixatives.

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(From Washington Square College, New York University, and the Marine Biological Laboratory, Woods Hole)

I. Purpose of the Study

A previous investigation of central bodies in the eggs of *Chætopterus pergamentaceus*, at metaphase, first cleavage, described the effects upon their structure of using various modifications of Boveri's picro-acetic reagent. It was found that central body configuration is closely related to the coarseness and shape of the astral rays, and that centrioles are demonstrated only when rays are very coarse and undulating (almost straight); this occurs only if the reagent is diluted with about 90 parts of water (Fry, 1932). Since such behavior of central bodies has not been reported elsewhere, and since those of *Chætopterus* have heretofore been regarded as typical centrioles, giving genetic continuity to successive division figures (Mead, 1898 and Wilson, 1930), it was thought desirable to determine whether or not the results produced by modifying a picro-acetic mixture hold true when diverse reagents are employed.[1]

II. Methods

For the most part, the methods of the present investigation were the same as those of the earlier study. The eggs used were taken from many females, obtained at different times in the season during the past four summers, at the Marine Biological Laboratory at Woods Hole; they were handled as previously described. As before, eggs of the various sets were left in the reagents from 6 to 15 hours (it was previously shown that these differences do not affect astral types when Boveri's picro-acetic reagent is used, and it is assumed that the same

[1] This study was completed by the aid of a grant from the National Research Council.

situation probably holds true in the case of other reagents). Eggs were again sectioned at a thickness of 5 μ and stained with Heidenhain's hæmatoxylin, every effort being made to destain them all to the same "medium" extent, in order that no variations in central body structure would be introduced by differences in depth of stain. As before, study was confined to the mid-sections of the larger aster of metaphase first-cleavage figures. Also the same method was used to secure a random sample of the various metaphase astral types which may be present in any egg-set, and thus to avoid an unconscious selection of any class.[2] Cells were studied with the same optical set-up and analyzed with reference to the same list of points (Fry, 1932, pp. 150–152 and 154–155).

The major data of the present study are reported in Table I (pp. 211–215) and include: first, the reagents employed; second, the structure of the central body and rays (astral type) demonstrated in each case; and third, the accompanying cytoplasmic configuration. Additional tables present various analyses of the data.

The 116 formulæ used fall into several categories: (*a*) most of the standard fixing fluids; (*b*) various original reagents; (*c*) the components of the different fluids used separately (in each case the amount dissolved is that usually called for when that component is used in standard reagents); and (*d*) dilutions of various members of the preceding groups.[3] Formulæ are given in Table I only when they are not found in Lee's *Microtomist's Vade Mecum*, ninth edition; page references are given for those found in that work.

The usual method of writing formulæ makes it impossible to appreciate at a glance the relative amounts of the components present in different reagents, because the items are called for in such terms as the following: "4 cc. of a 2 per cent solution of osmic acid," "corrosive sublimate to saturation," "3 drops of acetic acid," "99 cc. of a saturated solution of picric acid diluted with two parts of water," "1 gram of potassium dichromate," and "100 cc. of 60 per cent alcohol." In the present study, therefore, in addition to reporting the formulæ in the usual manner there is also reported in each case the percentage by

[2] As previously reported (pp. 164–165), the exact manner in which eggs happen to be added to the reagent in the vial may involve the production of several astral types, as a result of uncontrolled dilution effects, occurring within the first second, when fixation takes place, and differing in various parts of the vial, depending upon how the egg suspension happens to mix with the fixative.

[3] The results obtained in the earlier investigation with Boveri's picro-acetic reagent, both full-strength and diluted, and with the two components used separately, are included here in order that the data may be compared with those of the present study.

weight of each component, as well as the total percentage by weight of all the solutes. This permits ready comparison of the similarities and differences between the various formulæ. The figures given for the percentages by weight are only approximately correct, because, regardless of the care with which the reagents are made up and the weights of their components calculated, small quantities of sea water are inevitably added to the reagent when the eggs are added, and the percentages of the components are thus modified to an unknown and uncontrolled degree.[4]

Reagents made of the same components are arranged in groups in so far as that is possible. This permits ready analysis of such relations as the following: Is the presence of a certain component favorable to the demonstration of a given astral type, regardless of its amount or the presence of other components? If not, is the presence of that component, provided it occurs in certain amounts, favorable to showing that astral type and what is the effect of other components in various amounts? Is the total amount of solutes present in each reagent a significant factor?

The abbreviations used for the components are as follows:

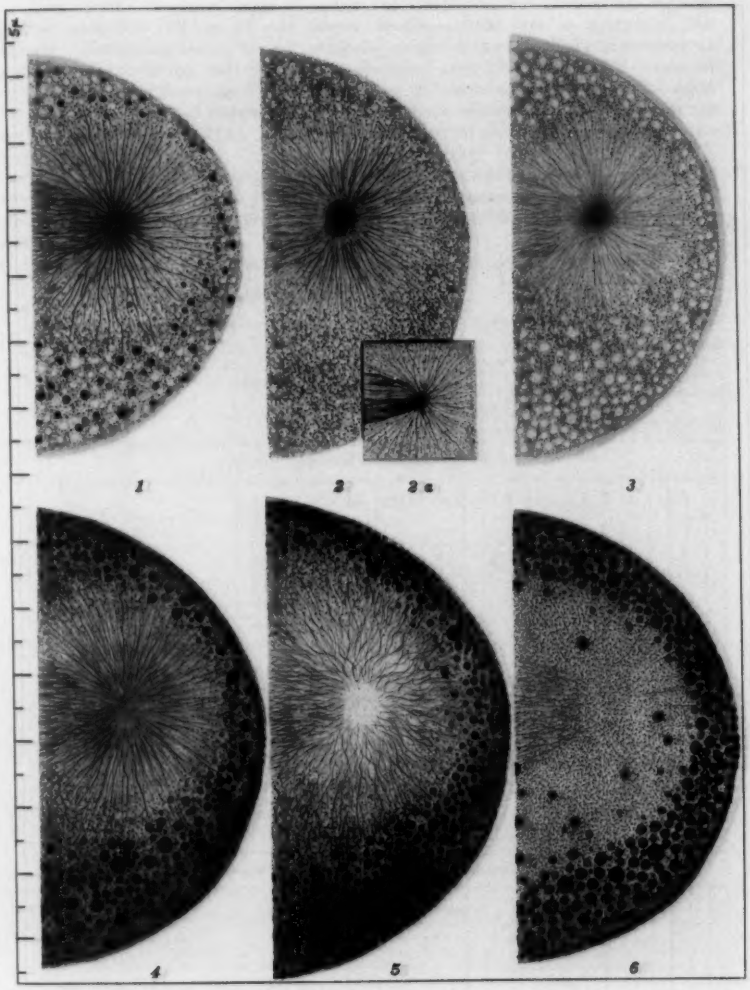
Acetic acid	*Act*	Osmic acid	*Osm*
Alcohol, absolute	*Alc*	Picric acid	*Pic*
Chromic acid	*Chr*	Platinic chloride	*Plt*
Chloroform	*Clf*	Potassium dichromate	*Pot*
Ether	*Eth*	Sodium sulphate	*Sod*
Formaldehyde	*For*	Sublimate, corrosive	*Sub*
Nitric acid	*Nit*	Sulphuric acid	*Sul*
		Urea	*Ure*

[4] Calculations of the percentages by weight were made as follows: (1) in the case of solids like chromic acid, the usual practice in preparing a 1 per cent solution is to add 1 gram to 100 cc. of water. While this is actually a 0.999 per cent solution by weight, it is here regarded as a 1 per cent solution. (2) Two solids, picric acid and corrosive sublimate, are commonly used in the form of saturated solutions: 1.22 grams of picric acid dissolve in 100 cc. of water at 20° C., and 6.5 grams of corrosive sublimate under the same conditions. In both cases the possible effects upon the amounts dissolved of relatively slight changes in room temperature and of the presence of other components were ignored, except in the case of the picro-sulphuric mixtures (formulæ 33 and 34), in which the sulphuric acid precipitates large amounts of the picric acid. (3) The percentage by weight of the fluids, glacial acetic acid, absolute alcohol, and ether, is determined by multiplying the number of cc. used by the specific gravity. (4) Chloroform is used as a saturated solution: 0.62 gram dissolves in 100 cc. of water at 20°. (5) The fluids which contain water—formaldehyde, nitric acid, and sulphuric acid—are treated as follows: (*a*) formaldehyde is assumed to be at 38 per cent strength in the average laboratory bottle (in contrast to the 40 per cent strength of a perfectly fresh solution), hence each cc. of fluid (sp. grav. 0.91) contains 0.34 gram of formaldehyde and 0.56 gram of water; (*b*) each cc. of nitric acid (70 per cent strength, sp. grav. 1.42) contains 0.99 gram of nitric acid and 0.42 gram of water; (*c*) each cc. of sulphuric acid (95 per cent strength, sp. grav. 1.84) contains 1.74 grams of sulphuric acid and 0.09 gram of water.

Explanation of Plate I

Each figure was drawn from a cell typical of its class. A little less than half of the cell is shown, enlarged 1000×. The symbols are those used in the tables.

Fig.	Reagent	Astral Type			Cytoplasmic Type			
		Central Body	Rays	Symbol	Peripheral Bodies	Symbol	General Cytoplasm	Symbol
1	Boveri's picro-acetic, 10% strength (formula 41)	Centriole with slightly demarked centrosome	Very coarse, undulating	5 *A*	Unstained, with few exceptions	*Un**	Rough	*Ro*
2	Acetic acid, 0.5% sol. (formula 3)	Slightly demarked centrosome	Medium coarse, undulating	4 *B*	Absent	*Ab*	Rough	*Ro*
2a	Chloroform-acetic (formula 72)	Ascaris-like central body	Medium coarse, undulating	4* *B*				
3	Kahle's formol-alcohol-acetic (formula 12)	Undemarked centrosome, dense	Delicate, undulating	3 *C*	Unstained	*Un*	Intermediate	*In*
4	Boveri's picro-acetic (formula 37)	Undemarked centrosome, even	Medium coarse, undulating	2 *B*	Stained	*St*	Intermediate	*In*
5	Boveri's picro-acetic (formula 37)	Disrupted	Medium coarse, rippled	1 *B*	Stained	*St*	Intermediate	*In*
6	Champy (formula 84)	Undifferentiated	Vague	0 *D*	Stained	*St*	Even	*Ev*


1
2
2a
3
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5
6

TABLE I

The various structural types of asters and cytoplasm demonstrated by different fixatives, used both full-strength and diluted, in Chætopterus eggs, at metaphase, first cleavage. Determination of the percentage by weight of the chemical components, as well as the abbreviation used for each, are discussed page 209; the symbols employed for both astral and cytoplasmic types, including the use of asterisks, are explained in the descriptive material opposite Plate I, and those concerning the cytoplasm are further discussed page 219. *Result:* centrioles are demonstrated only when the fixation also demonstrates very coarse and undulating (almost straight) rays, a condition which is always associated with a disrupted cytoplasm; this is accomplished only by a few reagents, made with certain organic components, diluted to about 10 per cent of their usual concentration. The data of this table are analyzed in various ways in succeeding ones.

	Reagent							Astral Type						Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm		
								5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*	*Ab*	*Un*	*St*	*Ro*	*Ta*	*Es*
	Group I. Acetic acid, Formaldehyde, Alcohol																		
		Act	*For*	*Alc*	*Others*	*Total*													
1	Acetic acid 1.0 % sol.	1.0				1.0				√	√				√	√			√
2	" 0.75% sol.	0.75				0.75					√				√	√*			
3	" 0.5 % sol.	0.5				0.5			√		√				√		√		
4	" 0.25% sol.	0.25				0.25			√		√				√		√		
5	" 0.1 % sol.	0.1				0.1	√								√		√		
6	" 0.01% sol.	0.01				0.01						√				√		√	
7	Alcohol 20% sol.			16.0		16.0								√			√		√
8	Alcohol, 50% sol. 99 pts. Acetic acid, 0.5% sol. 1 pt.	0.55		43.0		43.55								√		√			√
9	" 60% strength	0.33		25.8		26.13					√	√	√			√		√	
10	" 20% strength	0.11		8.6		8.71				√					√		√		
11	Formaldehyde 5% sol.		1.7			1.7							√				√		
12	Kahle (modified): 95% Alc., 15 pts; For., 6 pts; Act., 1 pt; water, 31 pts.	2.0	4.1	22.5		28.6				√					√			√	
13	" 20% strength	0.4	0.8	4.5		5.7				√					√				
14	" 10% strength	0.2	0.4	2.2		2.8			√								√		
15	" 5% strength	0.1	0.2	1.1		1.4	√							√			√		
	Group II. Acetic acid, Formaldehyde, Picric acid																		
		Act	*For*	*Pic*	*Others*	*Total*													
16	Picric acid, sat. sol. 1 pt. Water 2 pts.			0.4		0.4							√				√		√
17	Bouin (Lee, p. 63)	5.0	8.2	0.87		14.0						√					√		√
18	" 50% strength	2.5	4.1	0.43		7.0						√					√		√
19	" 30% strength	1.5	2.46	0.26		4.2						√					√		√
20	" 20% strength	1.0	1.64	0.17		2.8					√						√	√	
21	" 10% strength	0.5	0.82	0.08		1.4	√				√				√		√		
22	" 5% strength	0.25	0.41	0.04		0.7	√	√							√		√		
23	" 1% strength	0.05	0.08	trace		0.14		√							√		√		
24	" 0.1% strength	trace	trace	trace		0.01							√		√		√		
25	Bouin, made with sea water	5.0	8.2	0.87		14.0						√			√*		√		√
26	" 20% strength	1.0	1.64	0.17		2.8	√				√				√*		√	√	
27	" 10% strength	0.5	0.82	0.08		1.4	√												
28	" 5% strength	0.25	0.41	0.04		0.7	√								√		√		
29	" 1% strength	0.05	0.08	trace		0.14	√								√		√		

TABLE I (*Continued*)

	Reagent						Astral Type							Cytoplasmic Type				
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm	
							5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group II. Acetic acid, Formaldehyde, Picric acid (Continued)																	
		Act	*For*	*Pic*	*Others*	*Total*												
					Ure													
30	Bouin, modified, plus Urea (Carothers' formula)	10.5	5.13	0.91	1.0	17.0					√					√		√
31	Picro-Formol (Bouin without Acetic acid)		8.59	0.92		9.5						√√			√	√	√√	
32	" 10% strength		0.86	0.09		0.95						√√			√	√	√√	
					Sul													
33	Picro-Sulphuric (Lee, p. 56) (Mayer's formula)			0.4±	3.3	3.7						√				√	√	
34	" 25% strength (Kleinenberg's formula)			0.1±	0.8	0.9						√				√	√	
					Pot													
35	Regaud (Lee, p. 336), 100 pts. Acetic acid 5 pts.	1.04	6.7		2.3	10.0				√	√				√•	√		
36	" 10% strength	0.1	0.67		0.23	1.0				√	√				√•	√		√√
	Group III. Acetic acid, Picric acid																	
		Act	*Pic*		*Others*	*Total*												
37	Picro-Acetic (Lee, p. 56) (Boveri's formula)	1.0	0.4			1.4					√	√				√	√	
38	" 50% strength	0.5	0.2			0.7					√	√				√	√	
39	" 30% strength	0.3	0.12			0.42	√√								√√		√√	
40	" 20% strength	0.2	0.08			0.28	√√								√√			
41	" 10% strength	0.1	0.04			0.14	√√								√√			
42	" 5% strength	0.05	0.02			0.07	√				√				√√			
43	" 1% strength	0.01	trace			0.01							√		• • • •	√	√	√
44	Picro-Acetic, made with sea water	1.0	0.4			1.4					√	√				√	√	
45	" 50% strength	0.5	0.2			0.7	√√				√					√	√	
46	" 30% strength	0.3	0.12			0.4	√√											
47	" 20% strength	0.2	0.08			0.28	√√								√√			
48	" 10% strength	0.1	0.04			0.14	√√								√•			
49	" 5% strength	0.05	0.02			0.07	√•											
50	" 1% strength	0.01	trace			0.01							√		√√	√	√	√
					Alc													
51	Picro-Acetic (form. 37) 80 pts. Alcohol 20 pts.	0.8	0.32		16.0	17.12				√	√				√√			
52	" 50% strength	0.4	0.16		8.0	8.56				√	√				√√			√√
53	" 10% strength	0.08	0.03		1.6	1.71				√	√				√√		√	√√
					Clf													
54	Picro-Acetic (form. 37) with Chloroform to saturation	1.0	0.4		0.6	2.0				√					√•			
55	" 50% strength	0.5	0.2		0.3	1.0				√	√							
56	" 10% strength	0.1	0.04		0.06	0.2		√		√					√√			√√
					Eth													
57	Picro-Acetic (form. 37) 99 pts. Ether 1 pt.	1.0	0.4		0.7	2.1					√	√			√√			
58	" 50% strength	0.5	0.2		0.35	1.0				√	√	√			√√			
59	" 10% strength	0.1	0.04		0.07	0.2				√					√√		√	√√
					Plt													
60	Picro-Acetic (form. 37) 80 pts. Platin. chlor., 1% sol. 20 pts.	0.8	0.32		0.2	1.32				√√	√√					√√		
61	" 50% strength	0.4	0.16		0.1	0.66				√√	√√							
62	" 10% strength	0.08	0.03		0.02	0.13				√					√	√√		√√

TABLE I (*Continued*)

	Reagent							Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components													Peripheral Bodies			General Cytoplasm		
								5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*	*Ab*	*Un*	*Sl*	*Ro*	*In*	*En*
	Group IV. Acetic acid, Chloroform, Ether																			
		Act	*Clf*	*Eth*	*Others*	*Total*														
63	Ether 1% sol.			0.7		0.7								√			√		√	
64	Ether, 1% sol. 99 pts. Acetic acid 1 pt.	1.0		0.7		1.7		√*								√				√
65	" 50% strength	0.5		0.35		0.85		√								√		√		
66	" 10% strength	0.1		0.07		0.17		√							√			√		
67	Ether-Acetic (form. 64) 80 pts. Alcohol 20 pts.	0.8		0.56	*Alc* 16.0	17.36				√						√				√
68	Ether-Acetic (form. 64) 95 pts. Formaldehyde 5 pts.	0.97		0.67	*For* 1.7	3.3				√						√*				√
69	Ether-Acetic (form. 64) with Chloroform to saturation	1.0	0.6	0.7		2.3				√						√*				√
70	Ether-Acetic (form. 64) 80 pts. Platin. chlor., 1% sol. 20 pts.	0.8		0.56	*Plt* 0.2	1.56				√						√			√	
71	Chloroform, saturated solution		0.62			0.62								√			√		√	
72	Chloroform, sat. sol. 99 pts. Acetic acid 1 pt.	1.0	0.61			1.61		√*	√	√						√				√
73	" 50% strength	0.5	0.3			0.8		√	√	√						√			√	
74	" 10% strength	0.1	0.06			0.16		√	√	√						√		√		
75	Chlorof.-Act. (form. 72) 95 pts. Alcohol 5 pts.	0.95	0.58		*Alc* 3.94	5.47		√	√							√				√
76	Chlorof.-Act. (form. 72) 95 pts. Formaldehyde 5 pts.	0.95	0.58		*For* 1.7	3.23		√*	√							√				√
77	Chlorof.-Act. (form. 72) with Corros. sublim. to saturation	1.0	0.61		*Sub* 6.5	8.1								√			√			√
78	Chlorof.-Act. (form. 72) 80 pts. Chromic acid, 1% sol. 20 pts.	0.8	0.49		*Chr* 0.2	1.49							√				√		√	
79	Chlorof.-Act. (form. 72) 90 pts. Osmic acid, 2% sol. 10 pts.	0.9	0.55		*Osm* 0.2	1.65								√			√		√	
80	Chlorof.-Act. (form. 72) 80 pts. Platin. chlor., 1% sol. 20 pts.	0.8	0.49		*Plt* 0.2	1.49						√					√		√	

TABLE I (*Continued*)

	Reagent		Astral Type						Cytoplasmic Type					
	Formula	Per Cent by Weight of Components							Peripheral Bodies			General Cytoplasm		
			5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DB*	0 *DB*	*Ab*	*Un*	*St*	*Ro*	*In*	*Ev*
	Group V. Acetic acid, Chromic acid, Osmic acid, Potassium Dichromate, Platinic Chloride														
		Act	*Chr*	*Osm*	*Pot or Plt*	*Total*											
81	Burchardt (Lee, p. 42)	5.4	0.62		*Pot* 1.5	7.5				√	√				√	√	
82	" 10% strength	0.5	0.06		0.15	0.7				√					√	√	
83	Potassium dichromate 1% sol.				1.0	1.0						√			√	√	
84	Champy (modified) (Lee, p. 353)		0.39	0.39	1.2	1.98					√	√			√		√
85	" 10% strength		0.04	0.04	0.12	0.19					√				√		√
86	Flemming (strong) (Lee, p. 37)	5.2	0.75	0.4		6.35				√					√		√
87	" 50% strength	2.6	0.37	0.2		3.17				√					√		√
88	" 5% strength	0.26	0.03	0.02		0.3				√					√	√	
89	Meves (modif.): 2% Osm. sol., 4 cc.; 1% Chr. sol., 15 cc.; Act., 3 drops	0.79	0.39	0.37		1.55				√	√	√			√		√
90	" 50% strength	0.39	0.19	0.18		0.76				√	√				√		√
91	" 10% strength	0.07	0.04	0.03		0.15					√				√		√
92	Flemming, without Acetic acid		0.78	0.4		1.18					√				√	√	
93	" 10% strength		0.07	0.04		0.11					√				√	√	
94	Osmic acid 0.2% sol.			0.2		0.2					√				√		√
95	Chromic acid 0.2% sol.		0.2			0.2				√					√	√	
96	Platinic chloride 0.2% sol.				*Plt* 0.2	0.2				√			√			√	
97	Hermann (Lee, p. 39)	5.2		0.4	0.75	6.35				√					√	√	
98	" 10% strength	0.52		0.04	0.07	0.63				√					√	√	
99	Hermann, without Acetic acid			0.4	0.78	1.18						√			√	√	
100	" 10% strength			0.04	0.07	0.11						√			√		√
101	Platin. chlor., 1% sol., 99 pts. Acetic acid 1 pt.	0.99			0.98	1.97				√	√				√	√	
102	" 50% strength	0.49			0.49	0.98	√	√	√	√					√		
103	" 10% strength	0.09			0.09	0.19		√	√						√		
	Group VI. Acetic acid, Corrosive Sublimate, Nitric acid																
		Act	*Sub*	*Nit*	*Others*	*Total*											
104	Nitric acid 0.7% sol.			0.7		0.7				√			√				√
105	Corrosive sublimate, sat. sol.		6.5			6.5						√			√		√
106	Kostanecki and Seidlecki (Lee, p. 49)		3.1	1.4		4.4					√	√			√		√
107	" 10% strength		0.3	0.14		0.44									√		√
108	Corros. sublim., sat. sol. 99 pts. Acetic acid 1 pt.	0.94	6.04			6.9				√					√		√
109	" 10% strength	0.09	0.6			0.69				√						√	

TABLE I (*Continued*)

	Reagent						Astral Type							Cytoplasmic Type					
	Formula	Per Cent by Weight of Components												Peripheral Bodies			General Cytoplasm		
							5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*	*Ab*	*Un*	*St*	*Ro*	*In*	*Es*
	Group VI. Acetic acid, Corrosive Sublimate, Nitric acid (Continued)																		
		Act	*Sub*	*Nit*	*Others*	*Total*													
110	Gilson (Lee, p. 49)	0.4	1.9	1.5	*Alc* 4.7	8.5					√					√		√	
111	" 50% strength	0.2	0.95	0.75	2.3	4.2					√					√		√	
112	" 10% strength	0.04	0.19	0.15	0.47	0.85					√					√		√	
113	Perenyi (Lee, p. 36)			4.1	*Alc* 25.9 *Chr* 0.16	30.1				√						√		√	
114	" 10% strength			0.4	*Alc* 2.59 *Chr* 0.01	3.0				√					√*			√	
115	Zenker (Lee, p. 50)	4.6	4.4		*Pot* 2.2 *Sod* 0.87	12.0						√				√			√
116	" 10% strength	0.46	0.4		*Pot* 0.2 *Sod* 0.08	1.2				√						√			√

When a given component is present in most of the reagents of a group, its amounts are listed in a separate column; but when it occurs only sporadically, it is listed in a column entitled "others."

With two exceptions, all dilutions were brought about by adding distilled water to the reagent. In the case of Bouin's and Boveri's fluids, sea water was also employed (formulæ 25–29 and 44–50).

Reagents which are reported as being used in the usual way, i.e., at full strength, were actually used at about 85 per cent strength, since they were diluted with about 15 parts of eggs and their accompanying sea water at the time of fixation; (from 0.5 to 1 cc. of egg suspension was added to from 4 to 4.5 cc. of fixative in a vial). This situation occurs generally in the fixation of eggs, and, in the case of central bodies in *Chætopterus* eggs, the effect of so sl ght a dilution may be ignored; only extensive dilution—the addition c f more than 25 or 50 parts of water—produces significant changes.

The number of dilutions at which any given combination of components was employed varies considerably. The method of reporting dilutions is as follows: for example, "Bouin, 10 per cent strength" means that 10 cc. of Bouin's fluid was diluted with 90 cc. of water.

In the case of each variously-fixed egg-set only the minimum number of eggs was studied which was necessary to determine the major astral class or classes present. In cases where two or more types occur, no effort was made to determine accurately the relative percentages of each class, since this would have added nothing of significance for the present investigation, and would have necessitated the study of large numbers of cells. Hence, if the examination of 12 metaphase figures in any one egg-set showed that they belong to the same class, with at most one or two exceptions, no further eggs were studied. This is usually the case, and a check in the proper column of Table I indicates the astral type present; any exceptional cases were ignored. If two or more types were found, each in considerable numbers, about 20 eggs were studied, and two or more checks indicate the types occurring, again ignoring the exceptional cases.

Plate I illustrates the major structural classes of central bodies and rays, various combinations of which constitute the astral types; it also shows the different cytoplasmic configurations. The terminology and symbols employed for central bodies and rays are essentially the same as the ones used previously (Fry, 1932, Chart 7, p. 177)[5]; those referring to the cytoplasmic types are described later (p. 219).

III. Results

The Relation Between Central Body Structure and Ray Structure

The data of this investigation, reported in Table I, are in harmony with the results of the previous study. The central body area in *Chætopterus* eggs is very unstable, and exhibits different morphological types after the use of different reagents; each type is always associated with a particular shape and coarseness of rays, and centrioles are demonstrated only if rays are very coarse and undulating (almost straight). This relation is therefore not a phenomenon associated with

[5] One change in symbols has been made. Central bodies previously described as "undemarked, even," *i.e.*, stained like the ray area, may have rays which are medium coarse (type 2 *B*), delicate (type 2 *C*) or vague (type 2 *D*) (Fry, 1932, Chart 7). In the latter case, where the radial organization of the aster is doubtful, the central body area is entirely undifferentiated from the peripheral portion; but in the first two the large non-radial centrosome is clearly differentiated from the surrounding ray area, hence the type of center accompanied by vague rays which was previously called "undemarked, even" and was designated by the symbol 2 *D*, is now called "undifferentiated" and is designated by a new symbol, 0 (zero) *D*, which places it at the extreme end of the series (Plate I).

In order to save space in the tables, closely related astral types are grouped together. For example, since the class with an "undemarked centrosome, dense" may be associated with medium coarse rays (type 3 *B*) or with delicate ones (type 3 *C*), the two are grouped together as type 3 *BC*.

the use of particular selected reagents, but holds true in the case of the many diverse ones employed in this study.[6] The details of this relationship were discussed in the earlier paper (Fry, 1932, pp. 176–179).

In order to determine whether or not minor differences in the exact degree of the coarseness of the rays, which are designated in Table I as "very coarse" (type *A*), are related to differences in the centrioles, six representative egg-sets were chosen for study from the 16 in which centrioles are present. The results are presented in Table II. It is apparent that the rays must not only be very coarse if centrioles are to be demonstrated, but that the heavier they are the larger is the centriole.

Of the 16 formulæ which demonstrate centrioles, some of them do so in practically all of the metaphase figures, while others produce this result in only about half of them, the remainder showing centrosomes without centrioles (4 *AB*). In the sets where all figures have centrioles, most of the cells show typical very coarse rays, or rays even heavier than the typical ones; but in the sets where centrioles are shown only in about half of the figures the rays are usually less coarse, in some cases approaching the medium coarse condition of type *B*. In other words, it would seem that after using reagents which are capable of demonstrating centrioles at all, the percentage of metaphase figures containing centrioles increases as the rays increase in coarseness.

When centrioles are present they do not exhibit the similarity in structural detail from one metaphase figure to another which is associated with typical centrioles. In any one egg-set they vary somewhat in size; they are usually round, but occasionally they are irregular in shape; their contour is smooth in most cases, but rough in others; and the configuration in the large aster may differ from that in the small one of the same figure. Points of this sort cannot properly be considered in a study confined to metaphase, but they will be discussed in the next paper of this group, which will describe their behavior through all phases of various astral cycles. For the same reason, discussion of the phenomenon of the doubling of the centriole when the aster elongates, about the time of metaphase, will also be postponed to that paper.

[6] Possible exceptions to the usual astral types are produced by three reagents: acetic acid, 0.01 per cent solution (formula 6); chromic acid, 0.2 per cent solution (formula 95); and Gilson's fluid at 10 per cent strength (formula 112). They all demonstrate asters with disrupted centers, many of them with typical rippled rays, "medium coarse," or delicate, such as are associated with these centers (1 *BC*); but in some cases the rays are so coarse and straight as to approach the ray configuration associated with the presence of centrioles. Judging from the character of the fixation and the great variability from cell to cell, these three reagents barely permit coagulation, and the results are probably without significance.

Slightly demarked centrosomes without centrioles (4 *AB*, Plate I, Fig. 2) are demonstrated by 20 of the reagents. They differ in size, shape, physical structure, capacity to take the stain, and the extent to which they are demarked from the ray area, as illustrated in Figs. 1 and 2. Four of the fixatives (formulæ 49, 64, 72, and 76) demonstrate unusually small centrosomes, about 2.5 × 3 μ in size, in contrast to the usual measurements of about 5 × 6 μ. These centers are very variable in their reaction to the dye: many of them stain deeply and resist

TABLE II

The relation between coarseness of rays and diameter of centrioles in Chætopterus eggs at metaphase, first cleavage. The table shows six egg-sets in which centrioles are present, listed with respect to differences in the coarseness of their rays. "A" is the typical "very coarse" condition; "A+" indicates rays which are somewhat coarser and "A−" rays which are less coarse. Each centriole diameter reported is an average of 15 measurements in different cells.[7] *Result:* the coarser the rays are, the larger is the centriole.

Formulæ				Ray Coarseness	Centriole Diameter
No.	Components	Name	Dilution		
48	Acetic-Picric	Boveri	10% strength (sea water)	A+	0.6 μ
41	Acetic-Picric	Boveri	10% strength (distil. water)	A	0.5
15	Acetic-Alcohol-Formol	Kahle	5% strength (distil. water)	A	0.5
5	Acetic		0.1% solution	A	0.3
27	Acetic-Formol-Picric	Bouin	10% strength (sea water)	A	0.4
21	Acetic-Formol-Picric	Bouin	10% strength (distil. water)	A− or B	0.3

prolonged destaining, while others on the same slide fail to hold the dye. Most of them are rough and irregular in appearance, but about 10 per cent resemble to some extent the large centriole typical of *Ascaris* eggs (Plate I, Fig. 2*a*). Such centers are listed in Table I in the same column used for the typical large slightly demarked centrosome (4 *AB*) but are designated by an asterisk. In all probability

[7] Measurements were made with an ocular micrometer, under such optical conditions that the distance between its lines was 1.6 μ. By the use of a mechanical stage each centriole was placed between the two lines and its diameter estimated by the eye. Obviously such measurements are only approximately correct.

these *Ascaris*-like central bodies in *Chætopterus* eggs, which happen to present an unusually orderly appearance, are but one class out of many, although of no more or less significance than are the larger centrosomes, which are not regarded by cytologists as typical central bodies.

As in the previous study (Fry, 1932, p. 157), some of the "undemarked centrosomes—even" (type 2 *BC*) may contain granules similar to the smaller ones found in the cytoplasm, and certain configurations of them may simulate centrioles. But their rarity, their variation in size, number, and location, make it obvious that they are merely random granules, and they can be dismissed from further consideration.

The Relation Between Astral Type and Cytoplasmic Structure

There are two distinct areas in the cytoplasm of *Chætopterus* eggs —the zone of peripheral bodies, and the general cytoplasm (Plate I). The peripheral bodies occupy a zone about 15 μ wide around the edge of the egg; they are most abundant near the vegetal pole, and least abundant near the animal pole; they are relatively large, the average diameter being about 1.5 μ, although this measurement varies widely. When different fixatives are used this zone exhibits one of three major structural types: first, the peripheral bodies are absent (table symbol: "Ab"); second, they are numerous but remain unstained (table symbol: "Un") after the use of Heidenhain's hæmatoxylin and therefore resemble small vacuoles; (in a few cases where a limited number of the bodies take the stain they are indicated by an asterisk); and, third, the bodies are numerous and deeply stained (table symbol: "St") with Heidenhain's hæmatoxylin.

The general cytoplasm which lies between the peripheral zone and the asters also exhibits three major structural modifications after fixation: first, it has a rough (table symbol: "Ro") and disrupted appearance, as though it had cytolyzed prior to coagulation; second, its appearance is intermediate (table symbol: "In") between that of the preceding and following conditions; and, third, it is even (table symbol: "Ev") and homogeneous in appearance and is largely composed of minute granules which are near the limits of visibility.

Table III presents a resumé of this data from Table I, so arranged that two relationships may be analyzed: First, is there any relation between the appearance of the zone of peripheral bodies and that of the general cytoplasm demonstrated by a given reagent? Second, is there any relation between these various cytoplasmic configurations and the astral type produced at the same time?

As to the first of these relationships, in the six cases when the peripheral bodies are absent the cytoplasm is always "rough"; and of 82

TABLE III

The relation between the astral type and the accompanying cytoplasmic structure demonstrated in Chætopterus eggs at metaphase, first cleavage. The horizontal columns represent the possible combinations of types of the peripheral zone and the general cytoplasm demonstrated by the various reagents; the vertical columns represent the astral types. The numerals indicate the number of formulæ of Table I which demonstrate a given combination.[8] ***Result:*** centrioles, together with their accompanying very coarse and undulating rays, are demonstrated only when the general cytoplasm is "rough" and looks as though it has cytolyzed, and the bodies of the peripheral zone are absent or unstained.

Cytoplasmic Types		Astral Types						
General Cytoplasm	Peripheral Bodies	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
Rough	Absent	2	3	1				
	Unstained	2	2					
	Stained							
Intermediate	Absent							
	Unstained		1	3	3	1	1	
	Stained		1	1	8	6	8	3
Even	Absent							
	Unstained		4	8	5	1		1
	Stained		1		5	7	6	4

[8] Such tabulations as this are complicated by the fact that the different combinations of chemical components used are represented in some cases by but a single formula, when no dilution was employed, and by two or three or more in cases where dilutions were used. In order to equate these combinations as nearly as possible for the purposes of tabulation the following procedure was used: (1) the combinations which were used in one, two, or three formulæ in which the extent of the dilution differs widely are all reported. Where larger numbers of dilutions were used, only three are reported for each combination of components, as follows: acetic acid, formulæ 1, 3, and 5; Kahle's acetic-alcohol-formol mixture, formulæ 12, 14, and 15; Bouin's acetic-formol-picric fluid, formulæ 17, 18, and 21; and Boveri's picro-acetic reagent, formulæ 37, 38, and 41. (2) Bouin's and Boveri's reagents made up in sea water were not counted in making the tabulations. (3) The Meves formulæ (89–91) were omitted, because they contain the same components as Flemming's (86–88). (4) Where reagents demonstrate more than one astral type, only the one having the most condensed center and the coarsest rays, *i.e.*, the one most closely approaching the structure of type 5 *A*, is reported, and each formula is thus counted but once.

cases in which the peripheral bodies are present, either stained or unstained, the cytoplasm is "intermediate" or "even" in 78, or 95 per cent of them.[9] Thus it is apparent that the fixed structural types of the two cytoplasmic areas are related to each other.

The relation between the various cytoplasmic configurations and the associated astral type is equally clear: if centrioles with slightly demarked centrosomes (5 *A*) are present, the general cytoplasm is always "rough," and the peripheral bodies are either absent or unstained, never stained. But if the centers are uncondensed and not at all like typical central bodies (types 2 *BC*, 1 *BCDE*, and 0 *DE*), the cytoplasm is either "intermediate" or "even"—never "rough"; the peripheral bodies are always present, usually being stained.

It seems probable that the fixed cytoplasmic type which is most like the living condition is that in which the general cytoplasm is "even" and many bodies are present in the peripheral zone. This conclusion is based upon the fact that when the living egg is studied with a high-power water-immersion objective many peripheral bodies are seen, and the general cytoplasm presents a relatively homogeneous appearance. It will be noted that such reagents as those of Champy, Flemming without acetic, Meves, and similar ones, demonstrate "even" cytoplasm and numerous peripheral bodies, and that these fixatives are among those generally regarded by students of cytoplasmic structure as yielding results most like the living condition.

It is concluded, therefore, that the demonstration of centrioles in metaphase first-cleavage figures of *Chætopterus* eggs is associated with the simultaneous disruption of the cytoplasmic structure. In general, the poorer the cytoplasmic fixation is, and the more it deviates from its appearance in the living condition, the more condensed the center is, and the coarser and straighter the rays—and the greater the chances of the demonstration of a centriole.

The Relation Between Astral Type and Egg Volume

Table IV presents data concerning the demonstration of various types of central bodies in relation to the volumes of the eggs and asters. Whenever the astral center is condensed, no matter what its detailed structure is (types 5 *A*, 4 *AB* and 3 *BC*), the average dimensions of the egg are $63.5 \times 70\ \mu$, and the volume is approximately 147,000 μ^3. But if the center is not condensed (types 2 *BC*, 1 *BCDE* and 0 *DE*), the

[9] The type of cytoplasmic structure described as "intermediate" resembles the "even" condition far more than it does the "rough" one. It is also to be noted that the character of the cytoplasmic fixation may vary not only from one egg-set to another supposedly fixed in the same manner, but also from one egg to another within the same egg-set.

average dimensions are 70 × 78 μ and the volume is about 200,000 μ³. Since the average diameter of the living egg is 97 μ, and its volume about 477,000 μ³, it is apparent that the technique brings about extensive shrinkage in all cases. However, in the eggs which are least shrunk, whose volume is about 40 per cent of the living condition, the centers are uncondensed, whereas in those shrunk to about 30 per cent of the volume of the living egg the centers are condensed, and some of them have centrioles.

TABLE IV

The relation between astral type and egg volume, in Chætopterus eggs at metaphase, first cleavage. For each astral type there is reported the number of differently fixed egg-sets studied, the average dimensions and volumes of the eggs (based in each case on the measurement of about 50 eggs chosen at random, in about equal numbers, from the different egg-sets), and the average diameters and volumes of the associated asters (these latter measurements being based upon about 25 eggs in each case). Astral measurements are only approximately correct, since it is impossible to determine accurately where the zone of rays ceases. *Result:* condensed centers, some of which contain centrioles, are found only among eggs which are shrunken to a maximum degree as a result of the technique.

Astral Type	5 *A*	4 *AB*	3*BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
Number of Egg-Sets Studied	4	7	7	7	8	8	5
Dimensions of Egg (μ)	63×70	61×68	66.6×72	69×75	73×80	69×82	68×76.4
Volume of Egg (μ³)	143,913	132,207	166,865	186,571	222,752	203,935	184,585
Diameter of Aster (μ)	31	28	31	30	33	Asters Vague or Absent	
Volume of Aster (μ³)	15,565	11,469	15,565	14,107	18,776		

In all classes of asters with distinct rays, the average diameter of the aster is 30.6 μ and the volume about 15,000 μ³. Variations in the different classes are relatively minor, and are probably not significant.

The Chemical Conditions of Fixation Necessary to Demonstrate Centrioles

Centrioles are demonstrated only if the chemical components required for their fixation are present in much smaller amounts than those of standard reagents, a condition brought about by dilution.—Among the reagents reported in Table I, 26 were used both at 100 per cent and at 10 per cent strength, or thereabouts. When used full-strength, not one of them demonstrates a centriole, and only four (formulæ 64, 72, 75, and 76) show centrosomes without centrioles (4 *AB*). The results of using

them at 10 per cent strength are varied: in 12 cases there is no dilution effect;[10] in the remaining 14, however, a more condensed center and coarser rays are demonstrated when the reagent is employed at 10 per cent strength instead of in the ordinary manner; these results are shown in Table V. Centrioles are demonstrated only after using a few diluted reagents.

TABLE V

The effects upon astral structure of diluting reagents, in Chætopterus eggs, at metaphase, first cleavage. Twenty-six reagents reported in Table I were used both at 100 per cent and at about 10 per cent strength. In 12 there is no dilution-effect. In the other 14 cases, however, there is a dilution-effect, here reported. *Result:* whenever there is any dilution-effect, the astral type demonstrated by the diluted reagents always has more condensed centers and coarser rays than that shown by full-strength fixatives. Centrioles are demonstrated only after the extensive dilution of certain fixatives.

Formulæ		Astral Types Demonstrated at 10% Strength and at 100% Strength						
Components	Form. No.	5 *A*	4 *AB*	3 *BC*	2 *BC*	1 *BC*	1 *DE*	0 *DE*
Act-Alc-For	12 and 15	10%		100%				
Act (1% sol.)	1 and 5	10%			100%			
Act-Pic	37 and 41	10%			100%			
Act-For-Pic	17 and 21	10%				100%		
Act-Clf-Pic	54 and 56		10%	100%				
Act-Alc-Pic	51 and 53			10%	100%			
Act-Pic-Plt	60 and 62			10%	100%			
Act-For-Pot	35 and 36			10%	100%			
Act-Eth-Pic	57 and 59			10%		100%		
Act-Alc	8 and 10			10%				100%
Act-Chr-Pot	81 and 82				10%	100%		
Act-Chr-Osm	86 and 88				10%	100%		
Act-Sub-Pot-Sod	115 and 116				10%		100%	
Sub-Nit	106 and 107					10%	100%	

[10] Formulæ 64–66 and 72–74 demonstrate type 4 *AB* regardless of the concentration used; 101–103 demonstrate type 3 *BC*; 97–98, 108–109 and 113–114, type 2 *BC*; 110–112, type 1 *BC*; 31–32, 33–34, 84–85 and 92–93, type 1 *DE*; and 99–100, type 0 *DE*.

The amounts of the components necessary for the demonstration of centrioles fall within narrowly prescribed limits.—Four different combinations of components demonstrate centrioles; each must be used at a specific range of dilution. The results, given in Table VI, show that when centrioles are demonstrated by these four combinations of components the total amount of solutes, regardless of what they are, is from 0.07 per cent to 1.4 per cent by weight. Above and below this optimum range other types of centers are produced, even when the same components are used.

Only certain organic components demonstrate centrioles.—Table VII presents all of the formulæ of Table I in which the percentage by weight of the total solutes falls within the range from 0.07 per cent to 1.7 per cent—a slight extension of the previously described optimum range. They are classified in four groups: (I) those containing the organic components necessary for the demonstration of centrioles; (II) those containing the anæsthetics, chloroform and ether, combined in any way with the preceding components; these two groups include the formulæ which contain only organic components; (III) those containing both organic and inorganic components; and (IV) those containing only inorganic substances.

The results are as follows: *Of the various components used in this study only acetic acid, picric acid, formaldehyde, and alcohol, and only certain combinations of these, demonstrate centrioles.* Thirteen formulæ falling within the prescribed range of total amounts of solutes contain these components, but only four of them fix centrioles (Group I, Table VII). The exact amounts of the components in the various combinations are very important: for example, an acetic-formaldehyde-picric mixture (formula 21), in which the total solute weight is 1.4 per cent, demonstrates centrioles and centrosomes (5 *A*); but when the same components have a total weight of 0.14 per cent (formula 23) the centrosomes are without centrioles (4 *AB*). In this case the greater concentration of components is the more favorable for showing centrioles. On the other hand, an acetic-picric reagent (formula 41) with a total solute weight of 0.14 per cent shows centrioles and centrosomes, but if the total weight of these same components is 1.4 per cent (formula 37) the center is an "even" undelimited centrosome (2 *BC*). In this instance the lesser concentration of components is more favorable for demonstrating centrioles. When acetic acid is used separately the situation is similar: a 0.1 per cent solution (formula 5) shows centrioles and centrosomes (5 *A*), but a 1.0 per cent solution (formula 1) demonstrates large "even" centrosomes (2 *BC*).

TABLE VI

The amounts of components which must be present if centrioles are to be demonstrated in Chætopterus eggs at metaphase, first cleavage
The only four chemical combinations which are capable of showing centrioles were used at various dilutions (Table I). They are grouped here in three classes—those in which the amounts of components are too great for the demonstration of centrioles, those in which the amounts are optimum for that result, and those in which they are too small. *Result:* centrioles are demonstrated only when components are present within certain narrow limits; the total percentage by weight of all solutes present in a reagent must be between about 0.07 per cent and 1.4 per cent.

The Four Reagents Demonstrating Centrioles	The Amounts of Components Required to Demonstrate Centrioles																				
	Too Large							Optimum							Too Small						
	Form. No.	Act	Alc	For	Pic	Total	Astral Type	Form. No.	Act	Alc	For	Pic	Total	Astral Type	Form. No.	Act	Alc	For	Pic	Total	Astral Type
		%	%	%	%	%			%	%	%	%	%			%	%	%	%	%	
Acetic Acid	1	1.0				1.0	2 *BC*	5	0.1				0.1	5 *A*	6	.01				.01	1 *BC*
	2	0.75				0.75	2 *BC*														
	3	0.5				0.5	4 *AB*														
	4	0.25				0.25	4 *AB*														
Acetic-Alcohol-Formol (Kahle)	12	2.0	22.5	4.1		28.6	3 *BC*	15	0.1	1.1	0.2		1.4	5 *A*	No Data						
	13	0.4	4.5	0.8		5.7	3 *BC*														
	14	0.2	2.2	0.4		2.8	4 *BC*														
Acetic-Formol-Picric (Bouin)	17	5.0		8.2	0.87	14.0	1 *BC*	21	0.5		0.82	0.08	1.4	5 *A*	23	.05		.08	.008	.14	4 *BC*
	18	2.5		4.1	0.43	7.0	1 *BC*	22	0.25		0.41	0.04	0.7	5 *A*	24	.005		.008	trace	.01	1 *DE*
	19	1.5		2.46	0.26	4.2	1 *BC*														
	20	1.0		1.64	0.17	2.8	2 *BC*														
Acetic-Picric (Boveri)	37	1.0			0.4	1.4	2 *BC*	39	0.3			0.12	0.42	5 *A*	43	.01			.004	.014	0 *DE*
	38	0.5			0.2	0.7	1 *BC*	40	0.2			0.08	0.28	5 *A*							
								41	0.1			0.04	0.14	5 *A*							
								42	0.05			0.02	0.07	5 *A*							

TABLE VII

The effects upon astral structure of using various combinations of organic and inorganic components, in reagents containing from 0.7 per cent to 1.7 per cent of solutes, in Chætopterus eggs at metaphase, first cleavage. In each group of reagents there are given the formulæ which produce the various astral types, the chemical components of each, and the total percentage by weight of solutes. *Results:* centrioles are demonstrated only by four organic components, and only by certain combinations of them. The presence of anæsthetics and of inorganic components suppresses centrioles. In general, organic components produce coarser rays and more condensed centers than inorganic. (The method of tabulation was that reported in footnote 8, p. 220.)

Reagent Group	Astral Type Demonstrated by Each Reagent						
	5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*
I Those Containing the Organic Components Acetic, Alcohol, Formol, and Picric	5 Act 0.1% 15 Act-Alc-For 1.4% 21 Act-For-Pic 1.4% 41 Act-Pic 0.14%	3 Act 0.5% 23 Act-For-Pic 0.14%	53 Act-Alc-Pic 1.7%	1 Act 1.0% 37 Act-Pic 1.4%	38 Act-Pic 0.7%	11 For 1.7% 16 Pic 0.4% 32 For-Pic 0.95%	
II Those Containing the Anæsthetics, Ether and Chloroform, and Components of Group I		56 Act-Clf-Pic 0.2% 64, 65, 66 Act-Eth 1.7–0.17% 72, 73, 74 Act-Clf 1.6–0.16%	59 Act-Eth-Pic 0.2%	58 Act-Eth-Pic 1.0%	55 Act-Clf-Pic 1.0%	71 Clf 0.62%	63 Eth 0.7%

TABLE VII (*Continued*)

Reagent Group	Astral Type Demonstrated by Each Reagent						
	5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*
III Those Containing Both Organic and Inorganic Components		102 Act-Plt 0.98%	62 Act-Pic-Plt 0.13% 70 Act-Eth-Plt 1.56% 36 Act-For-Pot 1.0% 103 Act-Plt 0.19%	60, 61 Act-Pic-Plt 1.32–0.66% 80 Act-Clf-Plt 1.49% 82 Act-Chr-Pot 0.7% 98 Act-Osm-Plt 0.63% 116 Act-Sub-Pot-Sod 1.2% 109 Act-Sub 0.69%	78 Act-Clf-Chr 1.49% 112 Act-Alc-Sub-Nit 0.85%	34 Pic-Sul 0.9% 79 Act-Clf-Osm 1.65% 91 Act-Chr-Osm 0.15%	
IV Those Containing Inorganic Components Only				96 Plt 0.2% 104 Nit 0.7%	95 Chr 0.2% 107 Sub-Nit 0.44%	85 Chr-Pot-Osm 0.19% 92, 93 Chr-Osm 1.18–0.11% 94 Osm 0.2%	83 Pot 1.0% 99, 100 Osm-Plt 1.18–0.11%

TABLE VIII

The effect of the presence of acetic acid on astral structure in Chætopterus eggs at metaphase, first cleavage. Reagents are reported in pairs, which differ only in that one member of each pair contains acetic acid while the other does not; pairs 3 and 10 differ also in the presence and absence of alcohol. For each pair there is reported the formula number, the components present, and the total percentage by weight of the solutes. The presence of acetic acid is indicated by bold-face type. *Result:* reagents containing acetic acid usually demonstrate coarse rays and more or less condensed centers, and those without acetic acid do not; centrioles and their accompanying very coarse rays are demonstrated only when acetic acid is present.

Pair Nos.	Astral Type Demonstrated by Each Reagent						
	5 *A*	4 *AB*	3 *BC*	2 *BC*	*BC*	1 *DE*	0 *DE*
1	39 **Act**-Pic 0.42%					16 Pic 0.4%	
2	22 **Act**-For-Pic 1.7%					32 For-Pic 0.95%	
3	15 **Act**-Alc-For 1.4%					11 For 1.7%	
4		65 **Act**-Eth 0.85%					63 Eth 0.7%
5		73 **Act**-Clf 0.8%				71 Clf 0.62%	

TABLE VIII (*Continued*)

Pair Nos.	Astral Type Demonstrated by Each Reagent						
	5 A	*4 AB*	*3 BC*	*2 BC*	*1 BC*	*1 DE*	*0 DE*
6			103 Act-Plt 0.19%	96 Plt 0.2%			
7				108 Act-Sub 6.98%			105 Sub 6.5%
8				98 Act-Osm-Plt 0.63%			100 Osm-Plt 0.11%
9				9 Act-Alc 26.1%			7 Alc 16.0%
10					111 Act-Alc-Sub-Nit 4.25%	106 Sub-Nit 4.4%	
11						89 Act-Chr-Osm 1.55% 92 Chr-Osm 1.18%	

The presence of the anæsthetics, chloroform and ether, prevents the demonstration of centrioles.—An acetic-picric mixture (formula 41) with a total solute weight of 0.14 per cent demonstrates centrioles and centrosomes; but if this same combination, in slightly differing amounts, has either chloroform (formula 56) or ether (formula 59) added to it, the total amounts of solutes in both cases being 0.2 per cent, centrosomes without centrioles are shown (4 *AB* and 3 *BC*). Similarly, acetic acid used separately in a 0.1 per cent solution (formula 5) demonstrates centrioles and centrosomes, but if either chloroform (formula 74) or ether (formula 66) are added, the total solutes being 0.16 per cent and 0.17 per cent respectively, centrioles are not shown, only the centrosome being present (4 *AB*).

The presence of inorganic components prevents the demonstration of centrioles.—In general, the demonstration of dark and condensed centers is more likely to result from using organic components than inorganic ones. For example, if acetic acid is used in a 0.1 per cent solution (formula 5), the astral type produced has a centriole and centrosome, but the addition of platinic chloride, the total solute weight being 0.19 per cent (formula 103), or of chromic and osmic acids, the total solute weight being 0.15 per cent (formula 91), prevents the demonstration of both centriole and demarked centrosome, producing instead types 3 *BC* and 1 *DE* respectively. Of the 14 formulæ of Table VII which demonstrate the most condensed centers (5 *A* and 4 *AB*), 13 of them contain only organic components, the single exception involving the presence of platinic chloride in formula 102.

Without an exception, the demonstration of any kind of a dark and condensed center requires the presence of organic components in the fixative. Of 20 formulæ which demonstrate such centers (5 *A*, 4 *AB*, 3 *BC*), 15 contain only organic components, and the other five contain both organic and inorganic (Table VII).

The formulæ of Table I which have total amounts of solutes in excess of the range just reported were analyzed in the same manner. While none of them demonstrate centrioles, the general situation concerning the effect upon the fixed astral center of using organic components in contrast to inorganic ones is the same.

Acetic acid is the component most favorable for the demonstration of centrioles and condensed centers generally.—The data of Table I were analyzed with reference to a possible specific effect of the presence of each component, regardless of the occurrence of other components. The only result of significance is in the case of acetic acid. Among the formulæ listed in Table I are 11 pairs which are similar both as to the components present and the total amounts of the solutes; the

members of each pair differ only in that one contains acetic acid and the other does not (in two cases the difference involves the presence or absence of acetic acid plus alcohol). Table VIII reports the astral types demonstrated by these pairs of reagents.

With one exception the presence of acetic acid causes the demonstration of an astral type with a more condensed center than that produced by a similar reagent without acetic acid; the exceptional reagent (pair 11), which is a chrome-osmic mixture, disrupts the centers whether acetic acid is present or absent. Of the 11 reagents which contain acetic acid, nine demonstrate more or less condensed "filled" centers, three of them with centrioles, and only two have disrupted centers. On the other hand, of the 11 similar reagents which do not contain acetic acid, only one demonstrates a "filled" center, and that is of the least condensed type (2 *BC*); the remaining ten have either disrupted or undifferentiated centers. Furthermore, it is significant to note that acetic acid is the only component which occurs in all four of the combinations which demonstrate centrioles; in one of them it is used alone and in the other three it is combined with other components.

Centrioles are more readily demonstrated if sea water is used to dilute the reagent in place of distilled water. Of the four combinations of components which demonstrate centrioles when diluted with distilled water, two were also diluted with sea water—Bouin's acetic-formol-picric mixture and Boveri's acetic-picric fluid. In both cases the full-strength reagents were made up with sea water, the picric acid being dissolved in it (which may possibly have affected the amount which went into solution). With both reagents the following results hold true: First, when sea water is used centrioles are demonstrated by a less dilute mixture (compare formulæ 17–24 with 25–29, and 37–43 with 44–50). Second, when sea water is used centrioles usually occur in all metaphase figures within the range of dilution which shows them at all; only about half of them show centrioles when distilled water is employed (Table I). Third, sea water dilution demonstrates a somewhat larger centriole than does distilled water (Table II). Finally, in the case of Bouin's fluid the use of sea water widens the range of dilution within which centrioles are demonstrated; this does not hold true of Boveri's reagent, although the position of the range is slightly shifted (Table I).

IV. Discussion

The previous study in this group (Fry, 1932) showed that in *Chætopterus* eggs at metaphase, centrioles are demonstrated only by a few of the many modifications of Boveri's picro-acetic fluid used, and only when the reagent is so diluted that the chemical components are

present in certain small amounts. The present study confirms and extends that conclusion: centrioles are demonstrated by only four different chemical combinations, out of 48 employed; certain organic components must be present, and the usual reagents must be diluted with 70 to 95 parts of water.

Although these facts appear to be well established, their interpretation is difficult, if not impossible. If many different combinations of components demonstrated centrioles when used in small enough amounts, the situation would be simplified, for we might then be dealing with the results of poor fixation, brought about by using reagents in amounts so small as to barely permit coagulation; but the fact that only four combinations are effective when diluted is hard to explain. If the four organic components which demonstrate centrioles had any characteristic in common which might be of significance for coagulation—if, for example, they were all reducing agents, or related acids—an explanation of the situation might be suggested, but such is not the case. Perhaps the result is due primarily to the presence of acetic acid, since it can demonstrate centrioles when used alone, and is present in the four combinations which show them; but why then should it be effective only when used in certain small amounts, and not when the slightest increase is employed (formulæ 1 to 6)? [11]

The fact that there is a relationship between the demonstration of a given type of central body and the simultaneous demonstration of certain specific configurations of other cell structures is not affected, however, by the inability to explain the coagulation phenomena in physico-chemical terms, and it is quite possible that an understanding of this relation may add to our knowledge of central bodies.

The previous investigation showed that the demonstration of centrioles in *Chætopterus* eggs requires the presence of very coarse, undulating rays, but that conclusion was based upon the use of various modifications of a single reagent, picro-acetic. The possibility that other fixatives might have different effects has been eliminated by the present study, in which the relation between the structure of the central body and that of the rays is found to be constant in all respects after the use of 116 formulæ of diverse chemical composition.

[11] The various components were analyzed in terms of their molar solutions, but the results were not significant. The fact that dilution with sea water is more favorable for the demonstration of centrioles than dilution with distilled water may be due to a difference in the hydrogen ion concentration (cf. Fry, 1932, pp. 171–172). Differences in the osmotic pressure of the reagents probably is not a factor, since complete fixation occurs within the first second, and the plasma membrane is therefore instantly killed (cf. Fry, 1932, pp. 173–176).

Furthermore, this relation is now extended to include the type of cytoplasmic structure shown, as well as the size of the cell. If the coagulation is such that the fixed egg is shrunk to about 40 per cent of the volume of the living one, and the cytoplasmic structure is somewhat like that of the living condition, astral rays are usually either vague or delicate and the center is never condensed; but if the process of fixation shrinks the egg to about 30 per cent of the volume of the living one, and disrupts the cytoplasm, the rays are usually coarse and the center more or less condensed. Only under the latter conditions are centrioles demonstrated, and then but rarely.

The two interpretations discussed in the previous paper again present themselves—with more data now available on both. In the first place, we may be dealing with a centriole so exceedingly difficult to demonstrate, and shown under such unusual conditions of fixation, that its behavior in this respect constitutes a new chapter in the history of this cell component. For some reason, as yet unknown, its demonstration requires the fixation of rays which are at the same time undulating and very coarse—if either condition occurs without the other no centrioles are shown. According to this point of view it would be assumed that the chemical composition of the centriole and the rays differs so widely from that of the cytoplasm that their proper fixation necessarily disrupts the cytoplasm and shrinks the egg to the maximum extent. The unusual difficulty in demonstrating *Chætopterus* centrioles might then explain their variability in detailed structure from cell to cell under the rare conditions when they are shown.

The other interpretation of these phenomena assumes that the central area of the living aster is largely composed of the same kind of materials as are the rays; that diverse fixatives modify the detailed configuration of the entire aster; that the morphology of the fixed center is related to the way in which the peripheral ray area is coagulated. In this event, it is further assumed that when extensive dilution reduces the components of reagents to very small amounts, the fixatives are then approaching the lower limits of concentration which will permit any coagulation at all. When this happens, under certain chemical conditions the cytoplasm is disrupted, the cell is shrunken to a maximum degree, the ray configuration is unusually coarse, and the focal area of such rays is consequently unusually condensed. Under such conditions, the centers may take various forms: some resemble large slightly demarked centrosomes; some are smaller, stain deeply, and look somewhat like *Ascaris* central bodies; and in others the center looks like a typical centriole surrounded by a centrosome. But all of these may be "focal bodies" (Fry, 1932, p. 181), *i.e.*, the results

of the coagulation of areas where rays or spindle fibers converge. From this point of view, the variability in central body structure from one cell to another on the same slide is explained by the assumption that the exact morphology of any center depends upon the structure of the associated rays, which, in turn, depends upon the extent of the dilution to which the individual egg was exposed at the time of fixation (Fry, 1932, pp. 164–165). If this interpretation is correct, the class of fixed *Chætopterus* eggs which is most like the living condition has a homogeneous undisrupted cytoplasm, numerous peripheral bodies, and relatively delicate rays accompanied by a large uncondensed central area (2 *BC*, 1 *BC* or 0 *DE*, Plate I, Figs. 4, 5 and 6). In this event, the demonstration of centrioles in *Chætopterus* eggs is probably a result of such poor fixation that the egg is grossly distorted, and we are dealing with coagulation artifacts, which are especially likely to occur at areas of focalized rays or fibers.

Which of these two interpretations is most applicable to the data? The answer to that question depends upon our criteria for determining "good" and "poor" fixation. No single procedure can be followed because different cell components exhibit such varying structural stability after coagulation. For example, blepharoplast-centrioles and chromosomes are so similar from cell to cell at any given stage, even after using diverse fixatives, that to determine the class of coagulation products which most resembles the living condition presents no difficulty in many investigations. On the other hand, the situation is very different if one is studying the detailed structure of the cytoplasm, or the exact configuration of astral rays, or the morphology of the central body in many egg cells. The central bodies of *Chætopterus* eggs exhibit various structural types—so many as to constitute a continuous series extending from a condensed and delimited condition to an uncondensed or disrupted one. In such a case, how shall the "best-fixed" class be determined?

The usual procedure in cells where the central bodies exhibit considerable diversity of structure after fixation is to select as "best-fixed" those which contain minute sharply demarked bodies at the astral centers, resembling the central bodies frequently seen in spermatocytes. *If the demonstration of a centriole-like body at the astral center is proof per se that it actually is a centriole, such procedure is valid.* In that case it is unnecessary to report the relative number of cells which contain centrioles, and the other classes can legitimately be dismissed as "poorly fixed" without any attempt to discover why they differ from the "best" class, and without analyzing possible interrelations between the classes. Furthermore, such evidence may be considered depend-

able even when the demonstration of centrioles is the result of an unsuspected and uncontrolled modification of the technique, which makes repetition of the result impossible when the technique is used in the usual way, a situation which has occurred in the investigation of *Chætopterus* centrioles, and is discussed in the previous paper (Fry, 1932, pp. 149–150 and 162–164).

The danger in assuming that the demonstration of a centriole-like body proves the existence of a real centriole lies in the fact that the coagulation of focalized rays and spindle fibers may possibly result in the production of a structure which simulates a centriole but is an artifact. Since the chemical composition and physical structure of the center of an aster or the tip of an anastral spindle differs from the peripheral part, is it not possible that it may coagulate differently? Furthermore, it has now been demonstrated that the structure at the focal area is in some cases extremely variable, and that its morphology is related to that of the converging rays or fibers. This has been shown in several studies: in cytasters, sperm-asters, and first-cleavage figures of *Echinarachnius* eggs (Fry, 1928, 1929); in metaphase first-cleavage figures of *Chætopterus* eggs (Fry, 1932, and the present paper); and in the anastral mitotic figures of brain cells in *Squalus* embryos (Fry and Robertson, 1933). In the latter case, a relationship is also demonstrated between the structure of the mid-bodies and the detailed configuration of the fibers of the mid-region of the spindle which are focalized by the cleaving cell. On the basis of such data the suggestion has been advanced that when areas of focalized rays and fibers are coagulated, structures may be produced which simulate central bodies but which are nothing but phenomena of focalization.

Difficulties of interpretation such as these explain the attitude expressed in various quarters—that the study of fixed cells frequently yields data of little or no value, due to the distortion of cell structure frequently resulting from coagulation, and to our lack of knowledge of the physical chemistry of the process. In a measure this attitude is justified; certainly no one would study fixed cells, for the reasons just given, if the desired information could possibly be secured by studying living cells. But in many cell types the structure in question is discernible only vaguely or is totally invisible, and the only possible method of investigation at present available is the use of fixed, sectioned, and stained material. To discard the method because of its dangers is to forego securing significant facts which can be obtained in no other way. Such a method is properly used, however, only when adequate steps are taken to guard against its dangers, and the precautions required differ in the case of the various cell structures.

The data presented in this and the preceding study of *Chætopterus* central bodies are still too incomplete to permit a decision as to whether they are atypical ones or focal artifacts, because the work has so far been confined to metaphase, first-cleavage figures. The next paper on this subject will describe the behavior of *Chætopterus* centrioles through all of the maturation stages of the egg, and from the early history of the sperm-aster through late cleavage. Some of the asters are large and show marked changes in ray configuration during a single mitotic cycle, elongating during the process; others are small and show but slight structural changes. The behavior of the centriole under these various conditions provides significant additional evidence concerning its nature.

V. Resumé

The structure of central bodies in *Chætopterus* eggs at metaphase, first cleavage, was studied after fixation with 116 reagents, which represent 48 different combinations of chemical components; all were used at the usual concentrations, and most of them at various dilutions.

Centrioles are demonstrated by only four of the combinations, composed of one or more of the organic substances, acetic acid, picric acid, formaldehyde, and alcohol. The only component present in all four is acetic acid. The presence of the anæsthetics, chloroform and ether, as well as of numerous inorganic substances, prevents the demonstration of centrioles. In the case of the four effective combinations the components must be present within very small and narrowly limited amounts, differing in the case of each combination, and requiring a dilution of the usual reagent with 70 to 95 parts of water. Dilution with sea water is more effective for the demonstration of centrioles than the use of distilled. The data of the present study are in harmony with those of the previous one, which was confined to modifications of a picro-acetic mixture.

As in the previous work, various types of central bodies are demonstrated, each associated with a specific astral configuration, which varies as to the coarseness and shape of the rays. Centrioles are shown only when rays are very coarse and undulating (almost straight), which occurs but rarely.

The demonstration of centrioles and very coarse undulating rays is always accompanied by a disrupted cytoplasm which looks as though it had cytolyzed before it coagulated.

After the use of all reagents the eggs are extensively shrunken. Those which are about 40 per cent of the volume of the living egg have

uncondensed centers; those shrunk to about 30 per cent have condensed ones and some of these contain centrioles.

When centrioles are present they do not exhibit the orderly behavior of typical ones. In some egg-sets they occur in practically all metaphase figures; in others they are found in about 50 per cent of them. They differ, from one metaphase figure to another on the same slide, in size, shape, contour, and other points. The larger centrosomes also vary in every respect; after the use of a limited number of reagents, about 10 per cent of them look somewhat like the central body typical of *Ascaris* eggs.

The two interpretations discussed in the previous study again present themselves, but with additional data now available. On the one hand, we may be dealing with a very atypical centriole, different from any previously described, which can be demonstrated only under rare and peculiar conditions of dilute fixation, and only when a specific ray configuration is also shown; the cytoplasm is disrupted and the egg is shrunken to a maximum extent when this condition is produced. On the other hand, all of the various classes of central bodies demonstrated may be phenomena of focalization; the manner in which the peripheral part of the rays is coagulated may determine the morphology of their focal area; and when the conditions of fixation produce very coarse rays, the "focal body" is correspondingly condensed, happening sometimes to simulate a centriole.

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STUDIES IN THE LIFE HISTORIES OF EUGLENIDA

III. The Morphology of Peranema trichophorum Ehrenberg, with Special Reference to its Kinetic Elements and the Classification of the Heteronemidæ

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Peranema trichophorum (Ehrenberg) emend Stein is perhaps the most common of the larger, free-living, holozoic flagellates. It is easily cultivated in the laboratory, thriving on several media, and thus lends itself readily to experimental purposes.

The genus *Peranema* was established in 1841 by Dujardin, to receive organisms of the genus *Pyronema,* a term employed by him in 1836, but found to be already in use. He described three species, viz.: *P. globulosa, P. protracta,* and *P. virescens,* giving a figure of the first. Ehrenberg's description in 1838 is accompanied by nine figures which conform to the description of *P. protracta* by Dujardin, whereas the figure given by Dujardin does not resemble a *Peranema.*

Stein in 1878 placed the species *trichophorus* of Ehrenberg in the genus founded by Dujardin. He made an extensive investigation of the organism, describing a single flagellum and a single rod-organ. He also pictured the division of the animal. After him, a number of investigators, including Klebs (1883–1892), Fisch (1885), Butschli (1887), Senn (1900), Hartmann and Chagas (1910), Rhodes (1926), Hall and Powell (1928), and Brown (1930) recorded observations on the organism. The work of Klebs was especially careful. He described very accurately the mouth of the animal, two rod-organs, and a single flagellum. As a result of his studies, he divided the Euglenoidina of Butschli into three families: Euglenida, Astasiida, and Peranemida, which were respectively holophytic, saprophytic, and holozoic in nutrition. The Peranemida he divided into the subfamilies Peranemeæ, having a single flagellum, and Heteronemeæ with two flagella.

Senn changed this slightly in 1900, principally in establishing a number of subfamilies in his family Peranemaceæ. Dangeard in 1900 again instituted minor changes in terminology. Lemmermann, in 1913, followed Senn in naming the families, and Walton, in 1915, followed the same general scheme, although he traced the family names Eugle-

nidæ, Astasiidæ, and Peranemidæ to Stein, Butschli, and Ehrenberg respectively. Doflein (1916) retained the three families Euglenidæ, Astasiidæ, and Peranemidæ.

It will be noted that all classifications into families since Klebs have been largely dependent on modes of nutrition, symmetry perhaps playing a secondary part. Calkins in 1926 dropped the Peranemidæ. Those organisms possessing chloroplasts he placed in the family Euglenidæ, including here also a few questionable colorless forms. In the Astasiidæ, he included the colorless forms having a single flagellum. For colorless forms having two flagella, he created a new family, the Heteronemidæ.

Descriptive work on *Peranema trichophorum* shows an animal with a single flagellum and one or two rod-like structures in the mouth region. Dangeard (1901) shows two of these rods. Walton describes one; Lemmerman figures two, but speaks of a single parabasal body. Hartmann and Chagas show two, Hall and Powell two; Brown two; Doflein has a brief description of the family and the species, accompanied by an original figure. He shows a single flagellum, bifurcated at its base, the two roots ending in basal bodies, located against one of the two rod-organs ("stabapparat"). Calkins' description of the animal is largely in his key. He figures a single rod-organ, calling it a parabasal. Hall (1926) and Hall and Powell (1928) also describe the flagellum as single, and non-bifurcated at its base, while Brown (1930) shows it to be bifurcated at its base. Other workers describe the flagellum as single, vibratile at its tip, emerging from the mouth region and with a basal body. The animal is said to be spindle-shaped, tapering anteriorly, 20 to 70 microns long and 10 to 25 wide. It has a pronounced pharynx and reservoir into which a contractile vacuole opens. The thin pellicle is faintly striated spirally. Movement is creeping, although the animal swims at times. It frequently changes the shape of its body, becoming rounded up or forming an irregular ball. Nutrition is holozoic according to Walton and Lemmermann; saprophytic according to Doflein; and is not mentioned by Calkins.

While working on the life cycle of the animal (Lackey, 1929), it was noted that it appeared to have two flagella, so more careful cytological studies have been made.

Methods

The organisms were killed and fixed on coverslips which were either floated on the cultures or immersed until the animals crept on them. Schaudinn's fluid, Flemming's strong solution with and without acetic acid, and Bouin's fluid were used as fixatives. Of several stains em-

ployed, iron hæmatoxylin and Regaud's hæmatoxylin with eosin as an occasional counterstain were the most successful ones. Living animals were studied under oil in hanging drop preparations.

Findings and Discussion

A number of changes in the present description of the animal and some new characteristics which are regarded as diagnostic are enumerated. The length of the animal often exceeds 70 microns, specimens as long as 90 microns having been encountered. Individuals under 50 microns were not found, although many "wild" specimens were examined, in addition to those from the cultures. A very small form, 25 to 35 microns long and 10 to 12 wide, was often found in both stock cultures and material collected from various localities. While it appears to be identical with the larger form on visual examination, no mass cultures could be procured from isolations of it.

Bilateral symmetry is very evident; the mouth is on the ventral surface, and of peculiar shape, perhaps most comparable to a tuning fork whose prongs are short and pointed, the base of the fork being at the anterior end of the animal (Fig. 1). Klebs (1892) shows this very clearly; according to Hall (1926) the cytostome is subterminal.

Explanation of Plate I

Fig. 1. A diagrammatic sketch of the anterior end of *Peranema trichophorum* viewed from the ventral side showing the various organelles and their relation to each other. No connection between any of these and the nucleus has been found during interkinesis.

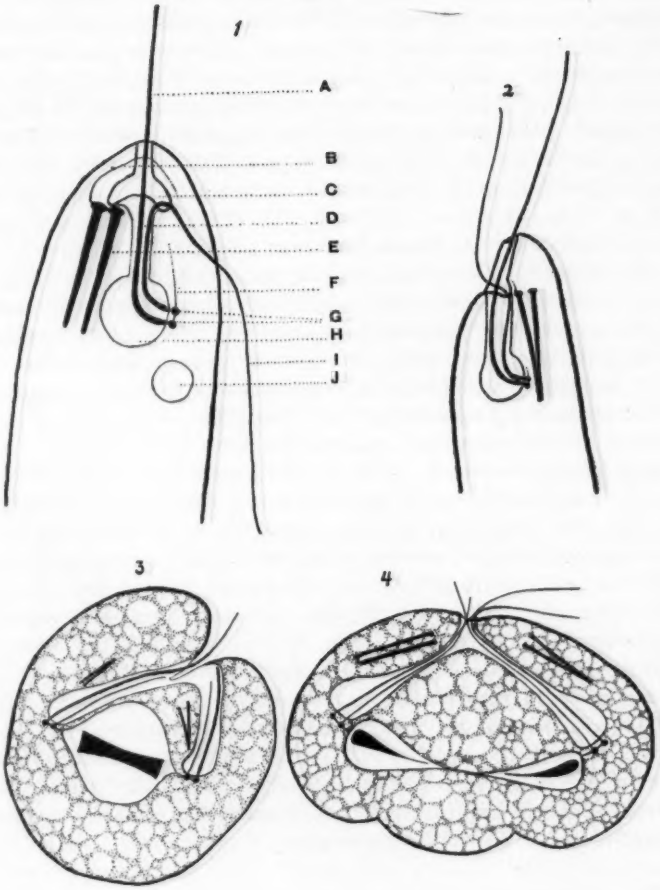
A. Primary, anteriorly directed or swimming flagellum.
B. Curved cytostomal element.
C. Mouth opening.
D. Internal gullet from mouth to reservoir.
E. Rod organs.
F. Fibril from blepharoplasts presumably to curved cytostomal element.
G. Two blepharoplasts.
H. Reservoir.
I. Secondary, posteriorly directed or trailing flagellum.
J. Contractile vacuole.

Fig. 2. A similar sketch of the anterior end of the animal as seen from the side. The two rods may either form a V or appear as single in such a view.

Fig. 3. A diagrammatic drawing of a *Peranema* about the metaphase in division. The cytoplasm has been stippled to bring out the nucleus and the gullet-reservoir more clearly. The chromosomes of the dividing nucleus are not shown, but the elongating endosome and the persistent nuclear membrane are shown. The nuclear membrane appears to be pulled out at the poles and is attached to the blepharoplasts, but there is no connection of any kinetic or cytoplasmic organelles to the endosome. Four rod-organs are seen to be arising *de novo* in the cytoplasm, the original pair having disappeared.

Fig. 4. A similar drawing of a telophase stage. The four flagella are clearly evident, having elongated sufficiently to emerge through the mouth. In this and the preceding figure each is seen to originate from one of the blepharoplasts.

PLATE I



The animal has two flagella. The existence of two has been unrecorded until now, although Lemmermann hints at the existence of a second (see Pascher; Die Süsswasserflora, Heft 2, Flagellatæ 2, page 117), and Conn and Edmondson figure an *Astasia trichophorum* with two flagella which may be *Peranema*. Failure to see the second flagellum is possibly due to a sublimate fixer, which often works havoc with delicate protoplasmic extrusions of this nature, whereas osmic fixatives leave them apparently intact. The larger flagellum is used for swimming or creeping, and only its distal quarter is vibratile. The smaller is a trailing flagellum and is about two-thirds as long as the body of the animal. Almost invariably it is held or trails naturally under the ventral surface of the body which often touches the substratum only at the sides, leaving an arch beneath the animal which would tend to hide the trailing flagellum. The writer has never been able to get more than a faint idea of its existence in living animals.

Both flagella emerge from the side wall of the reservoir (Figs. 1, 2), at right angles to the main axis of the animal. About the middle of the reservoir they turn sharply, paralleling the main axis, and pass up through the gullet into the mouth. The primary one extends normally straight out through the anterior troughlike portion of the mouth, so that in the living animal it appears to originate at the very tip of the animal. The secondary one emerges from one of the two arms of the mouth, turning backward. Both flagella have a very thick sheath as they leave the reservoir wall, but the primary is the larger. Both taper gradually, the sheath of the primary tapering to an end about where this flagellum becomes vibratile, while the sheath of the secondary is apparently lost in the gullet. The sheaths are either striated or uneven. The axial fibril of each flagellum terminates in a round blepharoplast adjacent to the reservoir wall. Hall (1926) (1928), speaking of a single flagellum (the principal one), says a rhizoplast is traceable to a granule which he terms the centrosome at the nuclear membrane. Hall and Powell state that this centrosome and not the blepharoplast serves as the pole in division. They find in the prophases a pair of granules or extranuclear centrosomes on the nuclear membrane connected by rhizoplasts to the blepharoplasts, and consider both blepharoplast and centrosome to be genetically continuous.

No amount of examination by the writer has shown a granule in the interphase on the nuclear membrane. There is often a vesicle above the nucleus, but no granule or rhizoplast in this region. Furthermore the old flagella are thrown off or absorbed in division and new ones arise, just as the writer has shown in *Entosiphon* (1929). This

is easily seen in the living animal and the writer has demonstrated it in stained preparations. The new ones grow out from two granules at each pole of the dividing nucleus, and these granules are the blepharoplasts. With the disappearance of the old flagella in the prophase the blepharoplasts each divide, and with nuclear elongation they migrate also, forming the poles of the dividing nucleus, as seen in Figs. 3 and 4. The animal is rounded up, although intensely metabolic, during division, and constriction of the cell does not begin until late; consequently the two gullets and reservoirs are close to the ends of the dividing nucleus. The heavy nuclear membrane remains intact and there is a definite orientation of the blepharoplasts as polar granules. But there is no origin of these granules from the endosome or any other part of the nucleus. These granules should therefore be termed centroblepharoplasts.

This account is in accord with that of Hall and Powell for one set of granules, but not for two sets. Nevertheless, it seems to the writer that to find a division of the blepharoplast and a division of an extranuclear granule which serves as a centriole or centrosome, and to assume a growth of a second rhizoplast (since one rhizoplast is found in the prophases by Hall and Powell) from the new centrosome to the new blepharoplast is a reductio ad absurdum. No division of the rhizoplast seems to be assumed, however. In reality there is no necessity for either assumption. The condition these writers show in Plate I, Fig. 4, is typical of the vegetative condition; nor do they show a separate blepharoplast and centrosome in any figures of their two plates, except possibly in Fig. 3. And as they show in the relative positions of gullet and dividing nucleus, only a very slight migration of the blepharoplasts would be necessary for these organelles to occupy polar positions. The actual occurrence is the division of the blepharoplasts in the prophase; the outgrowth of new flagella from them and their further functioning as extranuclear division centers, as shown by the writer in Figs. 3 and 4. They are wholly independent of the endosome in interkinesis and in division.

Brown (1930) shows very clearly the relationships of the blepharoplasts to the dividing nucleus in his figures. In his account he states that strands pass from these centroblepharoplasts to the endosome. From the number of these strands he shows, it might be concluded that they represent a spindle. In Fig. 6, pl. 19, for example, they are obviously invading the area occupied by the chromosomes as well. He is not clear on the mechanism of formation of a new kinetic complex: "During the prophase the basal granules divide twice to form new basal granules; out from these grow axial filaments. Each of these

filaments unites with one of the old axial filaments to produce a new flagellum. A part of the flagellum thus persists. . . ." If he means that *each* basal granule divides once, it is necessary to postulate a splitting apart of the two roots of the old flagellum. However, we have definitely stated that the old flagella are discarded in the early stages of division. If we accept the existence of a second flagellum, all accounts experience less difficulty in explaining their figures. Furthermore, it is hardly possible that Hall and Powell, Brown, and the writer would make such diverse observations with regard to the flagellum blepharoplast complex except on the basis of the existence of a hitherto unreported second flagellum. The evidence here, as elsewhere (see Hall and Powell), shows no nuclear origin for the kinetic elements, but instead a genetic continuity, which is to be expected for an organelle serving as a centriole and presumably homologous with the centrioles of higher forms.

There are also two rod-organs. They are practically parallel to the main axis of the animal, extending from about the region of the mouth to a point slightly below the reservoir. Often their posterior ends are close together, so that they form a narrow V. Like the blepharoplasts they stain well with iron hæmatoxylin. At the upper end each terminates in a slight knob alongside the gullet (Fig. 1). From the knob of one of them a thin fibril extends in a curve over the mouth and down in the general direction of the blepharoplasts. It has been impossible to trace the entire course of this fibril. It is probable that it is connected to one of the two blepharoplasts, since the distal blepharoplast has a faint fibril extending anteriorly from it, which may be the lower part of the fibril from the rod.

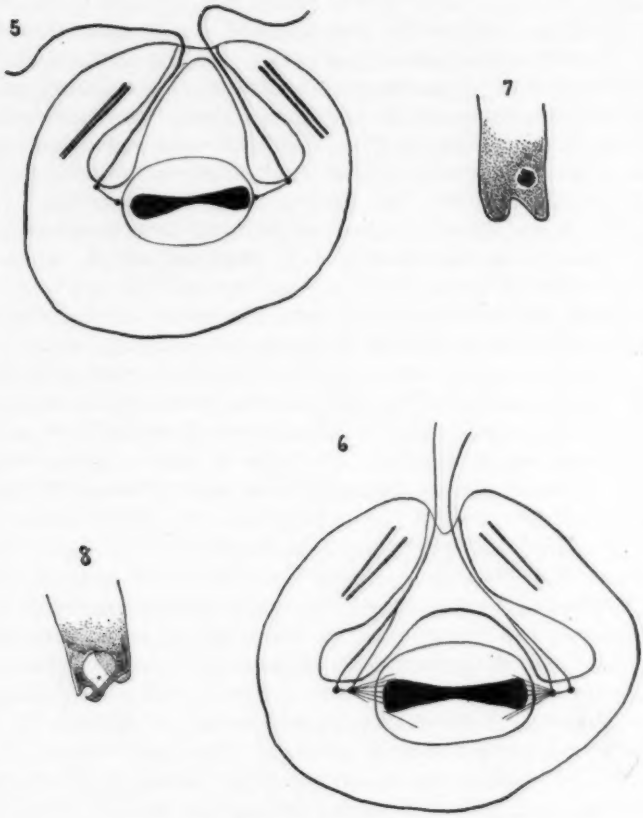
Rhodes (1926) finds similar rods (three or more) in *Heteronema acus,* which support the mouth and assist in food-getting. He says that Calkins is mistaken in calling the "staborgan" a parabasal body; that the "staborgan" is a true cytostome, and that Calkins is unjustified in creating the family, Heteronemidæ. *Peranema,* however, has but two rod-organs, and they appear to be morphologically similar. Neither of them has been observed to be contractile, as Rhodes says of the falcate rod in *Heteronema.* The writer has not observed that the rods of *Peranema* are of use in the ingestion of food, for while they are moved about in the metabolic activities that accompany ingestion, they remain close together, while the mouth stretches. Brown, however, has observed such a use. Schaeffer (1918) described "several" rods in *Jenningsia,* and they were in close relation to the mouth; he does not know of the use of them in feeding, although, by compression of the

animal under a coverslip, he could force three of them out into the mouth.

To justify the use of the term parabasal as applying to these rods, there should be traced a connection between them and the blepharoplasts. To do this beyond the possibility of a doubt has been impossible so far, but the case seems practically proved, by means of the fibrils referred to (Fig. 1). Dangeard (p. 291) regarded them as probably analagous to the tube in *Entosiphon sulcatus,* as does Brown. He thought they were probably united by " une pièce en forme de fer à cheval." This horseshoe-shaped piece he shows to be in the same location as the cytostomal element described by Hall, and the fibril the writer has endeavored to trace to the blepharoplasts, and while the tube of *Entosiphon* has three thickenings, it is not connected to the blepharoplasts. Calkins (p. 91) has a figure of *Peranema* which does not wholly agree with the findings of the writer, but which shows the connection clearly; if the two parabasals are in side view they would appear as figured by him. Lemmermann shows a connection between the rods and the basal granule of the flagellum. A similar connection is very clearly shown in his figure of *Urceolus cyclostomus* (Stein) Mereschk. Schaeffer (1918) shows a similar fibril from the anterior end of one rod proceeding towards the base of the flagellum in *Jenningsia.* Parabasals are generally said to originate from the nuclear material. Hall and Powell do not positively give the origin of the rods in their work on *Peranema,* nor do they claim the rods to be parabasals. In a large number of division figures studied by the writer, the old rods are seen to fade out gradually and new ones are formed *de novo,* in the region between the pole of the dividing nucleus and the new mouth (Fig. 3), which is in accord with Brown's findings. There is no evidence whatever of a nuclear origin nor of an origin from the blepharoplasts. Such knowledge would not justify calling them parabasals, even if they are connected to the blepharoplasts. Rather they may be looked upon as organelles of the mouth region, similar to those in *Jenningsia* and *Heteronema,* possibly used in feeding.

The posterior end of the animal often presents an amœboid appearance. At times a clear vacuole may be observed there, as in Fig. 8, but more often vacuoles filled with foreign matter are seen. These occasionally liberate their contents directly through the posterior end of the animal. Changes of shape in the posterior end are too slow to be called amœboid, and the region is best spoken of as a defecatory area, perhaps similar to that in *Anisonema.* What modification of the pellicle exists here is not known.

PLATE II



EXPLANATION OF PLATE II

FIG. 5. Diagram of the structure of a dividing *Peranema* according to Hall and Powell and reconstructed by the writer from a study of their work. The flagellum is pictured as single and the second blepharoplast of the writer is interpreted by them as a centrosome.

FIG. 6. Diagram of the structure of a dividing *Peranema* according to Brown and reconstructed by the writer from a study of his work. The flagellum is pictured as single but bifurcated at its base, and each of the two roots ends in a blepharoplast, the nearer one at each pole of the dividing nucleus serving as a centrosome or centriole.

FIG. 7. Posterior end of *Peranema* showing a vacuole containing matter for defecation. Drawn from life.

FIG. 8. Posterior end of *Peranema* showing a clear defecatory vacuole. Drawn from life.

Nutrition is certainly holozoic at times. Hall and Powell have described the formation of food vacuoles. In a culture which contains *Entosiphon,* these latter are often swallowed; as many as three have been observed distorting the shape of a *Peranema.* Often there are food vacuoles in the mid-region of the animal, which contain objects resembling bacteria, and ingested diatoms have been seen. But in old cultures the animals will become clear and show no food vacuoles; from which it may be inferred that saprophytic nutrition is possible. The statement of Brown that Euglenoida are necessary for food is incorrect. Perhaps the correct nutritive condition is an heterotrophic one.

The above characteristics seem to the writer to be diagnostic. They warrant taking the genus out of the family Astasiidæ, and transferring it to the family Heteronemidæ. They afford further justification for dropping the Peranemidæ as a family, as Calkins has done. Neither nutrition nor symmetry present sharp enough lines of demarcation to divide the order into families; furthermore, the nutrition of the colorless members of the order is too imperfectly understood and too subject to change. Above all, the studies show that until each member of this group is carefully investigated, their classification and relationships cannot be regarded as fixed.

Summary

1. In addition to the usually described diagnostic features, *Peranema trichophorum* shows other and undescribed features as follows:

A. The ventral mouth begins as a short straight trough, then widens into a backward curving crescent.

B. There is an additional trailing flagellum emerging from the posterior part of the mouth and passing back beneath the body.

C. There are two rods of unknown function alongside the reservoir-gullet.

D. The blepharoplasts serve as division centers.

E. The posterior end of the body is a defecatory area.

2. The possession of the trailing flagellum transfers *Peranema* from the Astasiidæ to the Heteronemidæ, and justifies the reclassification of Calkins, whereby the family Peranémidæ was dropped.

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ONE STEP IN THE DEVELOPMENT OF HEREDITARY PIGMENTATION IN THE FISH ORYZIAS LATIPES[1]

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Studies by Aida (1921 and 1930) on the inheritance of the fresh-water fish *Oryzias* (*Aplocheilus*) *latipes* have shown that the various color effects may be referred to the action of genes as indicated by the following formulae:

Brown (wild type)	*B B R R*
Blue	*B B r r*
Red	*b b R R*
White	*b b r r*
Variegated on red	*B′ B′ R R*
Variegated on white	*B′ B′ r r*

The genes *B*, *B′*, and *b* control multiple allelomorphs and are autosomal and *R* and *r* are sex-linked. The development of these characters was studied by Goodrich (1927) with special reference to the effects controlled by genes *B*, *B′*, and *b*. The gene *B*, which is dominant to both *B′* and *b*, permits the maximum development of the melanophores which become uniformly distributed over the body surface. *B′* is dominant to *b* and causes variegation. In this case there are two types of melanophores present. One form is dark colored, having the normal development of melanin, and is irregularly distributed, producing the mottled appearance of the fish. The other type is found in areas not occupied by the darker form and it contains so little melanin that it is usually invisible. It may be detected, however, by treatment with adrenalin (Goodrich, 1927) or by other methods which will induce a concentration of the previously diffusely distributed melanin and thus render the cells visible. In the recessive condition (gene *b*) very few of the deeply pigmented melanophores can be found. Practically all are of the light or "Farblose" (Schuberg, 1903) variety.

In attempting to analyze this situation the question may first be raised as to whether intracellular or extracellular factors are critical

[1] This study is part of a program dealing with the development of Mendelian characters and color patterns in fish which is made possible by grants from the Denison Foundation for Biological Research at Wesleyan University. The writer desires to acknowledge the assistance given by C. B. Crampton and Rowena Nichols.

in determining which type of melanophore will be developed. It has been previously pointed out (Goodrich, 1927) that an extracellular factor such as a circulating hormone seems improbable. Such a hormone would be nearly uniformly distributed throughout the body and similar cells would be similarly affected. This then, acting alone, could not be the critical factor in the production of a variegated pattern. It seems therefore reasonable to suppose that the differential factor lies within the cells.

As the two types of melanophores apparently differ only in the amount of pigment produced the factors involved in melanin production have been investigated. For this purpose the so-called dopa reaction (Bloch, 1917) has been utilized. It is well known that tyrosine is the chromogen which is the precursor of melanin. Dopa is 3:4 dihydroxyphenylalanine, which is considered to be the first transformation product in the change from tyrosine to melanin. It therefore may be used as a test for the presence of such an oxidase. For further details of the chemistry of the dopa reaction Raper (1928) may be consulted.

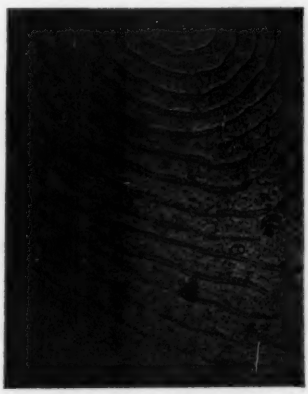
In these experiments scales have been removed from the yellow (red) fish and from light areas in variegated fish and treated with dioxyphenylalanin.[2] The methods used were those outlined by Laidlaw (1932). The method is simple and the results clear. In from two to three hours after placing the fresh scales in a dopa solution there may be seen in each scale a few cells which are amœboid in form and were previously nearly invisible but have become densely blackened. Other types of tissue cells do not show this reaction. This transformation is shown in Figs. 1 and 2. These are microphotographs taken with a yellow light filter which is necessary in order to eliminate the xanthophores which would otherwise be almost indistinguishable in the photograph from the melanophores. Figure 1 shows a fresh scale from a yellow fish. The light colored melanophores have contracted slightly and are therefore faintly visible, but can in this picture be only surely identified by careful comparison with Fig. 2 which shows the same scale after treatment with dopa.

These results then seem to indicate that the "colorless" chromatophores and no other cells contain an enzyme, the dopa oxydase (Bloch, 1917), which is probably identical with tyrosinase and which is necessary for the formation of melanin. These cells were, however, lacking in a precursor of melanin and the treatment described above has substituted dopa for that precursor.

It seems, therefore, probable that during histogenesis both types of melanoblasts possess the necessary oxydase but in one—that one which

[2] The dioxyphenylalanin was obtained from the Hoffman La Roche Co.

is to produce the "colorless" melanophore—there exists an insufficient quantity of chromogen. The three phenotypes are then probably similar in the production of oxydase but differ in chromogen content. The chromogen is present in maximum amount in all chromatophores of the dark form (gene *B*), is present in minimal amount in all chromatophores of the light types (gene *b*), and in the variegated types it is present in maximum amount in some cells and in minimal amount in other cells. This condition in the variegated types provides an added problem which apparently can be solved only in terms of some sort of embryonic segregation. It seems probable, as has been previously suggested (Goodrich, 1927), that at an earlier stage of development a segregation of mesenchymal anlagen for the prospective pigmented and non-pigmented melanophores may occur and the cells so differentiated



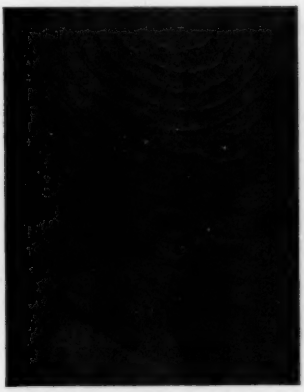


FIG. 1 (left). A microphotograph (× 120) of a part of a fresh scale from a yellow fish. The partially contracted light colored melanophores are almost invisible.

FIG. 2 (right). The same scale after treatment with dopa. Melanophores can now be readily located.

may become variously distributed by a process of amœboid migration characteristic of this type of cell. Such a period of segregation would mark the latest stage in development at which the critical cytoplasmic determination could occur in the variegated type and by inference it is to be expected that the cytoplasmic determination in the uniformly colored types would occur at a corresponding stage.

Dopa treatment has also been applied to the scales of the red and transparent forms of goldfish. In these fish (cf. Goodrich and Hansen, 1931) it is known that the melanophores disintegrate during an early

growth period and no evidence has been advanced to show the presence of "colorless" melanophores. The dopa test on these scales gave an entirely negative result. This gives further support to previous conclusions that the light phases of these two different species have been produced by very different developmental processes.

Conclusions

The light colored recessive phenotypes of the fish *Oryzias latipes* have been shown by use of the dopa reaction to possess the necessary oxydase for the formation of melanin. It is inferred that these differ from the dark colored dominant phenotypes in the relative absence of the chromogen tyrosine. Both chromogen and oxydase first appear intracellularly in the melanoblasts.

This condition is contrasted with that found in the goldfish where negative results were obtained.

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SCALE TRANSPLANTATION IN THE GOLDFISH CARASSIUS AURATUS [1]

I. Effects on Chromatophores. II. Tissue Reactions.

H. B. GOODRICH AND ROWENA NICHOLS

(From Wesleyan University and the Marine Biological Laboratory)

Materials and Methods

The existence of abundant variation in color and pattern in the goldfish, *Carassius auratus*, and the fact that the mode of color inheritance has been determined (Chen, 1928 and Berndt, 1928) suggested this fish as desirable material for a study of the development of inherited color patterns and the origin of pigment cells. Certain phases of the early development of the color pattern were studied by Goodrich and Hansen (1931). It was then felt that some information in regard to later phases of histogenesis and about the necessary tissue requirements of the different chromatophores might be obtained by a study of tissue transplants. The following five types of goldfish have been used for this purpose:

Brown or wild type of American breeders (normal-scaled)
Gold (normal-scaled)
Gold and silver (normal-scaled)
Transparent Shubunkin (transparent-scaled)
Ordinary Shubunkin or Calico Fish (designated "mottled" by Chen; largely transparent-scaled).

Chen (1928) describes the normal-scaled condition in goldfish as one in which all scales are backed with a layer of reflecting tissue. According to this classification the first three of the above varieties are normal-scaled. The types, gold, gold and silver (and the black and gold not used in these experiments) are also described by Fukui (1927) and Berndt (1928) as arising by a process of progressive depigmentation from the self-colored brown of the young fish (see Goodrich and Hansen, 1931). It seems clear that there occurs a permanent block to the process of depigmentation at different points in individual fishes.

[1] This study is part of a program, dealing with the development of Mendelian characters and color patterns in fish, which is made possible by grants from the Denison Foundation for Biological Research at Wesleyan University.

The cause of this block, whether hereditary or due to other factors, is at present unknown. It has, however, been shown (Chen, 1928 and Berndt, 1928) that the mottled fish or ordinary Shubunkin is a heterozygous type from the cross between the transparent Shubunkin and, apparently, any type having normal scales. Goodrich and Hansen (1931) have shown that the mottled and transparent types also arise by a process of depigmentation which begins much earlier in ontogeny than is the case with the first three named varieties.

The brown goldfish is a dark olive drab which grades ventrally to a silvery gray. All the scales are backed with reflecting tissue consisting of guanin crystals, and the dermis is abundantly pigmented with melanophores and xanthophores which together give a dull brown. The gold variety is likewise normal-scaled and the presence of numerous xanthophores in the dermis gives them their characteristic brightness. Great variation in color pattern ranging from gold with small silver areas to predominantly silver with small patches of gold exists in the gold and silver fish. Scales in the silver areas are without chromatophores and are therefore white in appearance, but the reflecting tissue shining through the scales gives a decided luster best described as silvery.

The transparent Shubunkin is largely without reflecting tissue. Absence of the guanin crystal layer renders the scales and tissues transparent, which undoubtedly led fish fanciers to give this form the name "scaleless." In this type we find marked variation in the amount and kinds of pigment. Sometimes chromatophores are entirely lacking and the individual is white, but more frequently there are a few deep-lying melanophores on the dorsal side of the body and in the region of the caudal peduncle. An occasional scale is backed with reflecting tissue and infrequently a transparent fish has a small area of xanthophores on the body or head.

The color pattern of the mottled fish or ordinary Shubunkin is much more complex than that of any of the fish already described. There is no uniformity in arrangement of colored scales nor those backed with reflecting tissue. A variegated design of black, yellow, and white, with a bright spot or two of gold and silver, characterizes this fish. Chen (1928) and Goodrich and Hansen (1931) give a more detailed description of goldfish varieties and an account of the variation of color pattern occurring in these forms.

The operation of transplanting tissue is very simple owing to the fact that the exposed part of the scale is covered with pigment-bearing dermis and epidermis, and removal of the scales carrying this tissue is easy. Transplantations of the skin of fish have been made by Šećerov

(1912). This method is probably impractical in goldfish and is much less convenient than the method of scale transplantation which seems to have first been used by Mori (1931). When removed, each scale leaves a small pocket in the dermis which facilitates the introduction of another scale, thus eliminating the necessity of an artificial means of attaching the transplanted tissue. Scales were removed from the host fish by means of a pair of fine forceps and the scales from the donor previously removed were tucked into the empty spaces. If more than a minute was to elapse between the removal of a scale and transplantation, the scale was kept in normal salt solution. Frequently there was a discrepancy between the size of scales exchanged and therefore the pockets they filled. It was found that by trimming the base of the scale to the desired size the edges of host and donor tissue could be brought into close proximity, thus insuring more successful healing of the transplant without any apparent injurious effects. For this operation and later observations on the scales the fish were anaesthetized with chloretone. A coördinate system utilizing the lateral line as a base was devised for locating all transplanted scales.

I. Effects on Chromatophores

Autoplastic Transplantation

Autoplastic transplantations, or transplantations to another part of the same individual, proved equally successful on all varieties of goldfish. Within forty-eight hours circulation was established on the scale and frequently the transplant had a functioning capillary net-work within thirty hours after the operation. In the case of autoplastic transplantation of pigmented scales a temporary contraction of the chromatophores occurred. Soon after the circulation was established, however, the cells were observed to be in the same condition as those on the rest of the body. This temporary contracted state is probably due to the failure of the cells to receive nervous impulses after the severing of the tissue (cf. review by Parker, 1930). Further investigation would be necessary to determine whether the contracted condition of melanophores on dark scales after transplantation approximates the stellate condition of denervated melanophores of *Fundulus* reported by Wyman (1924). There is no loss or change in the reflecting tissue when a scale is put into a new position on the same fish. In homoiotransplantations and those between different varieties of goldfish the reflecting tissue degenerates rapidly. Therefore this particular type of tissue serves as a very sensitive indicator of partial or complete success of a transplantation.

No degeneration of chromatophores or of reflecting tissue occurs in autoplastic transplantation in uniformly colored fish or in any other type except in young fish undergoing depigmentation. Other results, however, obtained from transplantations on the mottled Shubunkin and the gold and silver type have bearing on the problem of pattern formation.

In the mottled fish a black scale placed in either a white or gold region retains its melanophores. A light scale (white or gold) placed in a dark region assumes a complete complement of melanophores and often also of xanthophores, if they are present in the dark area. Melanophores first appear at the periphery and later invade the center of the scale, indicating a migration rather than a development in situ. Similarly, a gold scale placed in a white region retains its pigment and a white scale becomes invaded by xanthophores in a gold region. The migration of xanthophores is markedly slower than that of melanophores. In the ordinary Shubunkin after about ten days a light scale transplanted to a dark area has a complement of melanophores, whereas on a gold and silver fish approximately twenty-five days are required for a silver scale to become completely gold. The proliferation and migration of chromatophores varies markedly between individuals according to age and other undetermined factors; therefore, the above estimates of time are averages. A summary of the experiments is shown in Table I.

It has also been found that chromatophores will migrate from a transplanted scale to the surrounding region. Here again the activity of the melanophores is more pronounced than that of the xanthophores. Of 35 dark scales put into light areas on the mottled type of fish, 25 showed a spreading of melanophores sometimes to the extent of twice the diameter of the scale. The phenomenon occurs more frequently in scales transplanted into the lateral line, below it, or positions not more than two scale rows above it. The melanophores more frequently wander into the immediately sub-epithelial dermis but are sometimes found in the dermis lying beneath the scale.

The gold-silver fish proved more favorable than the mottled Shubunkin for testing the migration of xanthophores but the results in both cases are similar. Gold scales transplanted to light areas retained their color but xanthophores rarely migrated until after four months in the new location. After this time xanthophores were observed in the tissue surrounding gold scales but they had migrated not more than about one-fifth of the diameter of a scale.

In order to test the behavior of tissue that has recently lost a complement of melanophores, transplantations were made on young gold-

fish undergoing depigmentation. These fish are black and gold, the black area covering the dorsal aspect of the body. Fukui (1927) described the progress of depigmentation in the common goldfish as a wave of melanophore destruction that starts in the belly region and progresses dorsally. For further discussion of depigmentation of goldfish varieties, see Goodrich and Hansen (1931). Gold scales from the belly region or lower sides were exchanged with the more dorsal black scales. There was rapid healing of the tissue and the black scales lost their melanophores within two or three days. In two cases the fish turned gold so rapidly that no pigment change occurred in the gold

TABLE I

Autotransplants

Type of Fish	No. of Scales	Type of Transplant	Fate of Chromatophores
Gold	30	Gold on gold	No change in chromatophores.
Brown	32	Black on black	No change in chromatophores.
Shubunkin	41	White or gold on black	Assumed complement of melanophores.
Shubunkin	35	Black on white or gold	Kept melanophores, and in case of 25 scales there was a spreading of melanophores into the surrounding dermis.
Gold and silver	46	Gold on silver	Kept xanthophores, and in all cases there was a slight migration of these cells to the surrounding dermis.
Gold and silver	26	Silver on gold	Assumed complement of xanthophores.
Young goldfish (in depigmentation)	17	Black on gold	Lost all melanophores.
Young goldfish (in depigmentation)	20	Gold on black	17 assumed complement of melanophores.*

* On fish kept in cold room melanophores did not invade gold scales.

scales put into the dark region. In four fish, however, the process of depigmentation was slower and fifteen gold scales put into the dark region of these fish were completely covered with melanophores from five to seventeen days after the operation. Frequently, as one might expect, these scales lost the newly acquired melanophores within a few days, for the process of depigmentation overtook them even in their new position.

In order to retard depigmentation one fish was kept in a cold room.

Only after sixty-eight days were melanophores observed on two gold scales transplanted to the black area. The lower temperature not only inhibited depigmentation but also retarded the migration of melanophores from the surrounding dark area into the light scales.

Homoiotransplants

In all cases of transplantation between different individuals of the same or different varieties the chromatophores were found to disintegrate. This was preceded by the disappearance of reflecting tissue. In cases where the scales were placed in the self-colored types (wild or gold) they usually later assumed the complete host pigmentation. This is known to have occurred in 270 out of 343 successful transplants. In cases of transplantations into Shubunkins the results varied according to the pigmentation of the region that received the scale.

A complete tabulation and further details of these results will be given in Part II.

Discussion

The work of Chen and Berndt (*loc. cit.*) has indicated that the mottled type is the hybrid between the transparent Shubunkin and the "scaled" forms which include the brown, gold, gold and silver varieties in these experiments and which are therefore genetically similar in regard to the characteristic "scaled." The hybrid contains melanophores irregularly distributed and these may be practically absent from the adults of either homozygous parent. These experiments show that melanophores may live and multiply over any part of the surface of the body of the hybrid. Knowledge of the process of depigmentation (Fukui, 1927; Goodrich and Hansen, 1931) indicates that melanophores cannot exist for long periods in most adult goldfish. Melanophores transplanted from the diminishing black areas to gold areas disintegrated at once. The work of Goodrich and Hansen (1931) on the development of the transparent type also shows that in those forms some condition is present which destroys melanophores. Unfortunately, the existence of tissue antagonism (Part II), which prevents the successful transplanting of melanophores from one fish to another, does not permit a more direct analysis of the reaction of the homozygous types to melanophores.

The inference, however, seems fairly clear that while growth and persistence of melanophores is possible in the heterozygous type, some inhibition exists in the white homozygous form and sometimes (the complete gold type) in the other homozygous parent. The work of Smith (1932) has shown that melanin formation may be induced in gold types by the use of X-rays or by extensive de-scaling (Smith,

1931), both of which treatments may be considered as tissue irritants. The melanophores so produced, however, do not persist, which again indicates that tissue or physiological conditions are not favorable for them in these types. Unpublished experiments with X-ray treatment by Goodrich indicate that the transparent fish has less power to form melanophores than the gold type. Xanthophores, on the other hand, may be present in all types but in decreasing abundance as we pass from the scaled to the hybrid to the transparent forms. When, however, melanophores are in a favorable environment, they are far more active than xanthophores. These results are in agreement with the findings of Reis (1926) and Bock (1926) on Amphibia.

II. Tissue Reactions, Homoiotransplants

More than one thousand transplantations of tissue-bearing scales have been made between different fishes. The scale is imbedded in the dermis which normally contains all of the chromatophores and this is covered superficially by the non-pigmented epidermis. The transplant then consists of the scale and dermis and epidermis. In all cases the homoiotransplants have shown some degree of incompatibility or antagonism with the host tissues. In no case whatever have the donor chromatophores or the guanin crystals persisted. These have disintegrated and have been replaced by new chromatophores and guanin crystals if these were characteristic of the host. All possible exchanges between the four types of fish have been made, including transplants between fish of the same types.

Practically all of our fish have been obtained from one fish breeder,[2] and it is therefore probable that some of the transplants have been between closely related individuals (syngenesiotransplants in the terminology of Loeb, 1930). According to Loeb, less antagonism would be expected in such cases. In order to investigate the possibility that some of our fish might be so closely related that no antagonism would arise, transplants were made on a number of occasions between one gold type and five or six different gold type fish. Little of the extreme type of antagonism was noted, but in all cases the donor chromatophores disintegrated. Evidence in regard to the persistence of other tissues is discussed later.

The disappearance of chromatophores has, however, been the only constant feature of the homoiotransplants. There has been much variation in the rate of disintegration of the chromatophores, in the persistence of other dermal tissues, including the horny scale, and

[2] The fish have been obtained from the Grassyfork Fisheries, Inc., of Martinsville, Indiana. We are indebted to this firm for very careful attention to our orders and requests.

possibly in the persistence of the surface epithelium. A decided inflammation frequently occurs shortly after transplantation. None of these reactions has appeared in autotransplants made under conditions that were otherwise identical. The inflammation, if it occurs, usually arises within three days after transplantation and disintegration of chromatophores follows shortly. If there is no inflammation, the destruction of the chromatophores may be delayed until from ten to fourteen days after transplantation, which is also about the usual time of reëstablishment of the circulation, while in autotransplants the circulation may be established within thirty hours. New pigment cells appear at this time at the edge of the scales. The keratinous scales may be dissolved after four or five weeks. Observations have been continued for two or three months if transplants were not completely incorporated earlier.

Microscopic sections show a striking contrast between autotransplants and homoiotransplants. The former differ in no way from sections of normal untransplanted scales while the latter show a progressive thinning of the epidermis and a disintegration of the dermis. The chromatophores are the first cells observed disintegrating. Melanin granules pass to the surface epithelium and are sloughed off. Abundant leucocytes can be observed in fresh tissue. No clearcut edge of advancing host tissue such as that described by Rand and Pierce (1932) has been observed.

Homoiotransplants have been classified as follows on the basis of the various types of reactions: (1) transplants in which the donor scale is retained, (2) transplants in which the keratinous scale is dissolved to be later replaced by regeneration. This second group is further subdivided into those which show inflammation of the dermis and those which do not become inflamed. Inasmuch as no autotransplants have shown inflammation, it has been felt that this was the result of tissue incompatibility and not due to faulty technique. The donor chromatophores disintegrate in both classes. It is usually impossible to determine the fate of other dermal tissues.

Transplants are usually made by reciprocal exchange between pairs of fish and from three to five such exchanges being made per pair. The fate of the transplants on a given fish has been essentially the same. Hence it is obvious that it is a physiological reaction of the individual fish which is tested—not that merely of a given transplant. The results reported here are based on two years' work (1931–32) and on two separate lots of fish. The first lot included 840 transplants and the second 253 transplants. The second series was undertaken chiefly to fill in certain gaps in the first series. The results here em-

phasized are, however, based chiefly on the first and larger series (Table II) and are limited to those transplants of which it has been possible to keep a complete record from start to finish. This reduces the number in the first series to 695. The second series, Table III, differs from the first in being less complete, as certain classes are omitted; there were fewer cases of inflammation and a much larger record of scales dissolved. This may have been due to more careful attention given to this feature or the second lot may have come from a more inbred stock. If all the percentages in Table II are increased by an equal amount, some degree of correlation will be noted between the

TABLE II

Homoiotransplants. First series 1931–32.

Transplants	Number of scales	Scale dissolved		Scale incorporated	Percentage dissolved
		Rapid reaction Inflammation	Slow reaction No inflammation		
					per cent
Brown to brown.....	22	0	0	22	0
Brown to gold.....	18	0	0	18	0
Brown to Shu.....	15	0	0	15	0
Brown to trans.....	18	0	14	4	77.8
Gold to gold.....	85	9	0	76	10.6
Gold to brown.....	30	0	0	30	0
Gold to Shu.....	30	5	2	23	23.3
Gold to trans.....	55	5	8	42	23.6
Shu. to Shu.....	29	3	20	6	79.3
Shu. to brown.....	13	1	0	12	7.7
Shu. to gold.....	24	4	0	20	16.6
Shu. to trans.....	23	11	3	9	60.9
Trans. to trans.....	51	12	19	20*	60.8
Trans. to brown.....	67	0	0	67	0
Trans. to gold.....	102	9	14	79	22.5
Trans. to Shu.....	113	61	9	43	61.9

Shu. = shubunkin.
Trans. = transparent.
* 15 of these scales recovered in spite of severe inflammation.

two tables except in the case of the transplants between transparent and transparent. Observations on transparent fish are especially difficult and liable to error. It is felt, however, that the conditions of observation in the first lot were more uniform and that it is not legitimate to combine the results of both years' work in a single table. Sex of goldfish cannot be determined by external inspection except during the breeding season. Our records give no indication of a sex-differential in the reactions of the transplants.

Discussion

The fact that all homoiotransplants in goldfish show tissue incompatibility while autotransplants show no antagonism establishes the existence in fish of individuality differentials in the sense in which this term is used by Loeb. This class of vertebrates may then be removed from the list given by Loeb (1930), p. 583, which shows no individuality differentials.

If the percentages of occurrence of transplants in which the scales dissolved as indicated in Table II are rearranged (Table IV), certain relationships become apparent. It appears that with the exception of the transplants from brown to transparent these types of reactions are much less likely to occur when the brown form is either donor or host

TABLE III

Homoiotransplants. Second series 1932–33.

Transplants	Number of scales	Scale dissolved		Scale incorporated	Percentage dissolved
		Rapid reaction Inflammation	Slow reaction No inflammation		
					per cent
Brown to brown	26	0	11	15	42.3
Brown to gold	30	0	8	22	26.7
Brown to Shu.	32	0	10	22	31.2
Brown to trans.	29	0	8	21	27.6
Gold to brown	23	5	11	7	69.6
Gold to Shu.	10	5	5	0	100.0
Shu. to Shu.	25	0	25	0	100.0
Shu. to brown	27	4	12	11	59.2
Shu. to gold	12	0	4	8	33.3
Shu. to trans.	10	0	10	10	100.0

than in any other exchanges. It may be noted that the high figure given for transplants from brown to transparent is not substantiated in the second series and that no cases of inflammation were recorded. The type here referred to as "brown" is the dark colored form occasionally appearing in breeders' stocks which most closely resembles the true wild fish. It is highly probable that the other types have arisen by mutation and by hybridization from the wild stock. It then appears that transplants to or from the wild-like form are more likely to be successful than those between mutant types.

According to Chen (1928) the brown type is, in regard to the characters which he studied, to be considered as genetically identical with the gold type. It is probable, however, that most Shubunkins

are produced by crosses between the transparent and the gold types. It is therefore of some interest to note the relations existing between the gold and transparent types and their hybrid, the Shubunkin. These are shown in the portion of Table IV enclosed by the heavier line. It will be seen that the transplants involving the gold type are more successful than those limited to the white and transparent forms. The whole relationship seems to suggest that the further removed a type is from the characteristics of the wild stem form, the nearer it is to a condition of unbalance in its tissue relations and that this condition is revealed in transplants. This situation may possibly also be correlated with the conditions of greater variability in development in the transparent form and the Shubunkin when contrasted with the gold type as shown by Goodrich and Hansen (1931).

TABLE IV

Percentage of homoiotransplants from first series showing destruction of scales. (Scale dissolved).

Donor	Host			
	Brown	Gold	Shu.	Trans.
Brown	0	0	0	78
Gold	0	11	23	24
Shu.	8	17	79	61
Trans.	0	22	62	61

Loeb (1930) has emphasized the point that grafts from a parent strain to a hybrid show relatively slight antagonism, while a marked reaction is likely to occur when a transplant is made in the reverse direction. It will be noted that the results from the goldfish are not in accord with this generalization.

Autotransplants of skin have been found in various groups of animals to be generally successful and to show relatively little tissue antagonism (cf. review by Korschelt, 1931). In many cases there is evidence of an interpenetration of tissues between the graft and the host until an equilibrium is reached. This does not usually appear to involve any extensive destruction of tissues, but the results of Rand and Pierce (1932) on Amphibia indicate that caution is necessary in drawing conclusions from total preparations. The results here outlined in Part I from autotransplants, especially in the Shubunkin and in the gold and

silver type, give evidence of the interpenetration of tissues. This is shown by the migration of the chromatophores and closely parallels such results as those obtained by Sale (1913) and Seelig (1913) on guinea pigs. Šećerov (1912) found a less rapid penetration in autotransplants of the skin of the fish *Nemachilus barbatula* and a relatively slow or incomplete replacement of tissue in homoiotransplants.

Conclusions

Part I

1. Autotransplants on all types of goldfish succeeded without loss of any of the tissue elements, epithelium, dermis, or horny material.

2. Autotransplants on fish having two or more different colored areas showed that scales without pigment when put into either gold or black regions assume a complement of chromatophores similar to the surrounding area. Pigmented scales keep their chromatophores when put into an unpigmented region and there is a tendency for these pigment cells to invade the surrounding dermis. Melanophores were found to migrate faster than xanthophores and to wander farther from the graft.

3. In homoiotransplants of scales between individuals of the same or different varieties, the reflecting tissue and chromatophores degenerated. After the loss of donor pigment, however, many of the scales assumed the chromatophores of the host.

Part II

4. Some degree of tissue incompatibility is found in all homoiotransplants. This varies from that apparently causing only the disintegration of chromatophores to that bringing about the destruction of the whole scale and accompanied by inflammation.

5. A wide variation in degree of tissue antagonism has been observed even between similar pairs of fish. The following relations, however, seem to be indicated:

A higher percentage of tissue antagonism is usually observed when the less pigmented types (Shubunkin and transparent) are involved than when the more pigmented types (brown and gold) are used, and transplants with the brown type show less antagonism than any other exchanges.

The further removed a pair of individuals is from the characteristics of the wild form, the more likely it is that incompatibility will be found in transplants between them.

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THE DIFFERING EFFECTS OF DIFFERENT PARTS OF THE VISUAL FIELD UPON THE CHROMATOPHORE RESPONSES OF FISHES

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In two previous publications[1] I have discussed a problem which presents itself when we consider the chromatophore responses of fishes to their background, under various conditions of illumination. It has long been known that many fishes become extremely dark upon a black background and very pale upon a white one, while they assume intermediate shades upon backgrounds which are likewise intermediate. An interesting feature of this situation is that the response to a given background is largely independent of the absolute degree of illumination of the latter. A black background, in direct sunlight, will call forth the maximum degree of darkening, while a white background, in rather dim light, will call forth nearly or quite the maximum degree of pallor. In a gray container, too, the shade of the fish will approximate that of its surroundings, regardless of the intensity of illumination. It is obvious, however, that a neutral gray surface, brightly illuminated, may reflect far more light—specifically, far more *white* light—than a white surface dimly illuminated. Why then, does the fish assume a darker shade on the former than on the latter?

To answer this question, I believe, we must consider why we ourselves recognize a surface as black, white, or gray, regardless of the degree of illumination to which it is subjected. The answer is simple. The object in question is perceived in relation to the total visual field. When, on a dark night, we recognize a sheet of white paper as white, it is because we make a "correction," as it were, for the dimness of the general illumination. If, for any reason, we are misled respecting the degree of illumination to which an object is subjected, we may see a gray object as white, or vice-versa.

Does not the fish react in a manner quite similar to this? For flatfishes, I have already argued that the animal is controlled not by the absolute intensity of the light reflected from the substratum, but by the ratio between this and the source of illumination overhead.[2] In the

[1] Sumner, 1911; Sumner and Keys, 1929.

[2] Sumner, 1911. In this idea of reaction to a ratio, I was anticipated by Keeble and Gamble (1904).

more recent paper by Sumner and Keys (1929), it was shown experimentally that when the underlying surface was caused to be considerably lighter or darker than would normally result from the given apparent source of illumination, the fish became paler or darker, as the case might be, than the background in question. Similar experiments with catfish (Pearson, 1930) have confirmed these results, at least within a considerable range of illumination.[3]

Since, of course, the fish has no notion respecting the source of illumination nor any means of identifying this, we may say that it reacts to a ratio between light received from two different portions of the visual field. One of our present problems is to identify these two portions, if only approximately. How much of the total field belongs in the numerator of our fraction; how much in the denominator? How much acts in a positive sense, such that making it lighter or darker in relation to the rest causes the fish, likewise, to become lighter or darker; how much in a negative sense, such that increasing or decreasing its relative brightness calls forth converse changes on the part of the fish? While I have not been able to answer these questions in a quantitative way, I trust that some light is thrown upon the subject in the following pages.

In my earlier experiments with the small flatfish, *Rhomboidichthys* (=*Platophrys*) *podas* (Sumner, 1911), I showed that the bottom of the container in which the fish was kept was far more influential in determining the chromatophore responses of the latter than were its walls. This was surprizing, in view of the fact that the fish itself commonly covered a very considerable fraction of the bottom, and that it lay much of the time with its head fairly close to the vertical walls of the jar. In another flatfish, *Lophopsetta maculata,* while the influence of the bottom was predominant under ordinary conditions, that of the near-by side walls of a small container was much more pronounced than in *Rhomboidichthys.* This difference between the two species was due, perhaps, to the lesser degree of motility of the eyes of *Lophopsetta,* as compared with those of *Rhomboidichthys,* which are placed on movable stalks and enable the fish to look almost directly downward.

If the color changes of fishes have a bionomic value in relation to concealment, one would expect bottom-dwelling fishes such as flounders to be influenced primarily by the substratum on which they lie. In freely swimming fishes, on the other hand, which move at various levels through the water, one would not expect the substratum to exercise such a predominant influence.

[3] Earlier experiments by Mast (1916), with somewhat different methods, had given results which only partially harmonized with those here cited. Mast's procedure has been criticized by Summer and Keys (1929).

I have performed several experiments upon the common Pacific killifish, *Fundulus parvipinnis* Girard, in order to determine the relative influence of the bottom and walls of the small glass aquaria in which the fishes were kept. Ten medium-sized specimens were placed in each of two shallow, cylindrical glass aquaria, 25 cm. in internal diameter by 14 cm. deep.[4] In one of these, the bottom was painted black, the walls white; in the other the condition was reversed. At the close of two months (the definitive condition was doubtless reached in much less time), the following conditions were noted. The eight survivors in the black-bottomed, white-walled aquarium were distinctly darker and more opaque than those in the white-bottomed, black-walled one. This was made certain by pouring the two lots simultaneously into white containers and making the comparison immediately, before color changes occurred. It is evident that in this experiment the bottom had exerted more influence than the walls, despite the fact that the former had an area of 491 square centimeters and the latter an area (below the water line) of 589 square centimeters. It must be noted, however, that the walls were far from being without influence. Fishes in the white-bottomed container were distinctly darker than those kept in an all-white one, while fishes in the black-bottomed container did not become as dark as those in an all-black one.

In another experiment, carried out earlier, the bottom areas were much more restricted, in proportion to the walls. Small battery jars, 18 cm. high by 13 cm. internal diameter, were used. The water was 10 cm. deep. Here the ratio of bottom surface to wall surface below the water line was about 1 to 3. Since running water was not used, the fishes probably swam near the surface much of the time. Five jars of each sort were employed, each containing two specimens. Careful comparisons by two observers were made on several different days, the fishes on each occasion being poured simultaneously into all-white jars. In every case the fishes from the black-bottomed jars were darker.[5]

The experiments hitherto described are open to one objection, namely, that the visual field is continually changing with the movements of the fish, or even with the movements of its eyes. Thus areas of very different degrees of illumination may be imaged in turn upon the same region of the retina. If different areas of the retina have different potencies, in relation to their control over the chromatophores, the experimenter must devise a method by which each region may be subjected more or less continuously to the same stimulus. It is the chief

[4] The depth of water, controlled by siphon outlets, was here about 7.5 cm.

[5] It is only fair to mention that a later trial of this experiment, with only one jar of each type, gave a result which was not in accord with the preceding. Individual propensities to swim at different levels may account for these differences.

purpose of the present paper to discuss results obtained by the use of one of these methods.

Von Frisch (1911) described the results of some experiments in which he had covered the eyes of trout partially or completely with a mixture of vaseline and lampblack. This substance, he tells us, commonly remained in place for a quarter of an hour or less, after which it fell off.[6] In brief, von Frisch's findings were as follows:

(1) Covering both eyes completely gave varying results, but in the majority of cases a decided, though not maximal, darkening occurred.

(2) Covering one eye completely resulted in the fish's becoming extremely dark; thus, darker than when both eyes are covered. In the majority of cases the darkening of the body was unsymmetrical, the side opposite to the covered eye being the darker.

(3) Covering the lower half of both eyes likewise led to a very dark condition.

(4) Covering the upper half of both eyes, on the contrary, had no effect upon the coloration. The fishes (which, during the experiment, were on a white background) retained their pale shade.

(5) Covering the lower half of one eye led to a pronounced unsymmetrical darkening; covering the upper half of one eye gave no result.

Von Frisch's method was inadequate in at least two respects. In the first place, the duration of treatment which it allowed was hardly sufficient for definitive results. In the second place, it was not possible, as he himself admits, to be sure that the covering was completely impervious to light. Any foreign object of this sort is soon detached by the secretion of mucus on the eye, and it probably separates partially from the surface sometime before it actually falls off.

Early in 1931, the present writer commenced an analogous but much more extensive series of experiments. After considerable trial and error, the following method was developed. Since it may be of value to other investigators, it seems worth while to describe this rather fully.

Gelatin medicinal capsules (chiefly the "No. 0" size, 7 to 8 mm. diameter) were dipped repeatedly into a solution of celloidin of medium thickness, the solvent being allowed to dry somewhat between times. After several layers of celloidin were accumulated in this way, the desired pattern was painted upon the rounded end of the capsule[7] in India ink by means of a fine brush. This also was repeated a number of times

[6] Parker and Lanchner (1922) covered the eyes of *Fundulus heteroclitus* with opaque screens, produced by pouring over them a mixture of collodion and lampblack, held in position by threads which had previously been stitched around the margins. Such drastic treatment would, however, seem likely to interfere with the normal responses of the fish.

[7] Both the body of the capsule and the cover may be used for the purpose.

to give the necessary opacity. After allowing the ink to dry [8] the capsules were dipped several times more into the celloidin solution. It was highly important to lay on enough of this substance to make the final product sufficiently rigid. The celloidin was again thoroughly dried, after which the gelatin of the capsules was dissolved out in water at

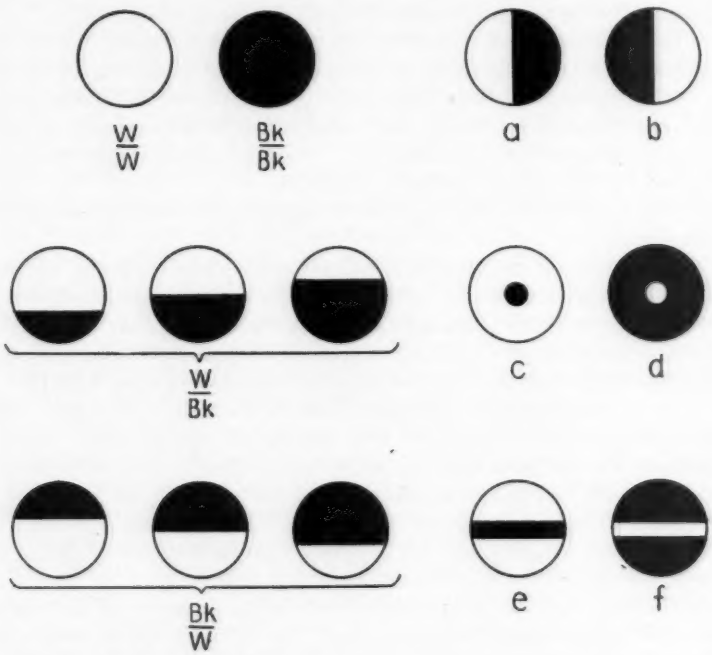

FIG. 1. $\frac{W}{W}$, $\frac{Bk}{Bk}$, $\frac{W}{Bk}$, and $\frac{Bk}{W}$, false corneas, wholly transparent, wholly opaque, and ones completely shading the lower and upper portions of the visual field (all uncolored). *a*, *b*, *c*, *d*, *e*, and *f*, additional types of "cornea" employed in a limited number of experiments. Dark regions completely opaque.

about 50° C. The celloidin coatings were now ready for trimming. A convex circular area was cut out from the end of each, in size somewhat larger than the exposed surface of the eye of the fish to be experimented upon.

In practice, these celloidin eye-caps, which I shall speak of as

[8] A drying oven at low temperatures was used for this and for drying the celloidin. The capsules were placed upon rows of pegs, consisting of finishing-nails driven into a board.

" corneas," were fitted into the front of the orbit of the fish, much as a glass eye is fitted into the orbit of a human subject. The fish was first anesthetized by being placed for a few minutes in a 1 per cent solution of urethane in sea water. No cutting of the tissues was necessary, though the conjunctiva was unavoidably lacerated somewhat by the edge of the celloidin cap. In most cases, these " corneas " remained in place for some hours at least, and frequently for a day or more.[9]

The accompanying diagrams (Fig. 1) show the types of uncolored " corneas " which I employed, i.e., ones upon which only black India ink (in the first case none at all) was used. Along with these diagrams are given the symbols for each which will be used in the ensuing text.

The $\frac{W}{W}$ was a cap of transparent, uncolored celloidin. The $\frac{Bk}{Bk}$ was intended to be completely opaque. In the later experiments, this point was tested by rather careful examination. The $\frac{W}{Bk}$ and $\frac{Bk}{W}$ were commonly divided into closely equal areas, so that when fitted into the orbit, the horizontal line of division passed approximately through the center of the pupil. Some were made, however, in which the opaque areas covered approximately one-third and two-thirds of the surface, respectively. When these were in position, the margin of the black area would just reach the pupil, in the one case, or just expose the edge of it in the other.

Certain other types of " cornea," which were used in only a few experiments, are represented on the right-hand side of the figure. The first two are obviously merely the $\frac{Bk}{W}$ or $\frac{W}{Bk}$ rotated through an angle of 90°. In *c* and *d*, the opaque spot and aperture, respectively, are of about the diameter of the pupil, while in *e* and *f*, the bar and the cleft are likewise intended to be of the width of the pupil. In practice, no such exact correspondence in dimensions was found possible.

Many experiments were also conducted with variously colored corneas. For this purpose Higgins's colored drawing inks were used. These, in thin layers, were found to be sufficiently transparent, though, needless to say, the character of the light thus transmitted was very imperfectly known. In the case of yellow, a much more satisfactory result was obtained later by using celloidin colored by picric acid. These

[9] In one case, they were removed after remaining seven days over both eyes. After a day or two, however, results cease to be very instructive, owing to injury to the eyes.

colors were used singly or in almost every possible combination with one another or with white (i.e., uncolored areas) or black. Thus I had $\frac{Y}{Y}$ (all yellow), $\frac{Bk}{Y}$ (black above, yellow below), $\frac{W}{Y}$ (colorless above, yellow below), $\frac{Bl}{Y}$ (blue above, yellow below), etc.

Experiments with these "goggles," as they were familiarly called, were commenced early in 1931, and have been conducted at intervals almost until the present time of writing. Altogether, several hundred of the "corneas" have been prepared, and records obtained from more than 200 fishes. As in the case of most biological experimentation which is carried beyond its initial stages, baffling contradictions and inconsistencies have been encountered, and for some of these no reconciliation has yet been found. This fact has been responsible for continual delay in my publication of these results, but present circumstances make it undesirable that I should postpone this report any longer.

The fishes here used were all of a single species, *Fundulus parvipinnis* Girard. Medium-sized or large specimens were used, ranging from 6 to 9 cm. in length. The sexes were not readily distinguishable during the winter and early spring, when most of these experiments were conducted. The effect of sex upon the color reactions at such times is very questionable. In the last of the series (May and June, 1933), females alone were employed, except for the purposes of comparison.

In a small proportion of cases, the fishes were tested before the experiments, in order to determine whether their chromatophore reactions were normal. This precaution was not generally taken, however, since otherwise healthy individuals seldom or never failed to respond in a striking manner to the shades of background here used. Much more frequently, a test of this sort was made after the fish had been subjected to experimentation. In some of these cases, the damage inflicted was found to have greatly impaired the animals' capacity for response.

I shall first consider those experiments into which the color element does not enter, i.e., ones in which the "corneas" have been coated with black ink or none at all. It may be useful to commence with comparisons between my own results and von Frisch's, in those cases in which we have performed analogous experiments.

In agreement with von Frisch, I found that when both eyes were covered with $\frac{W}{Bk}$ corneas—at least, when the upper and lower areas were approximately equal—the result was invariably a high degree of darkening on the part of the fish, regardless of the background on

which it was placed. This, indeed, was the most constant result obtained in the course of these experiments. The fishes (14 cases) were invariably much darker than controls, and were in most instances of nearly maximum darkness. At the same time, the scale pattern was commonly pronounced, owing to the expansion of the chromatosomes (Sumner, 1933) belonging to the melanophores which outline the scales. This is in contrast with the effects of some stimuli, which lead to the effacement of the scale pattern, even though the fish may remain fairly dark.

In a limited number of cases, as already stated, the shaded and unshaded portions of the "cornea" were not of equal extent. In two out of three cases in which the shaded area just reached the pupil, a less pronounced darkening resulted than when the field was equally divided. In two out of three cases in which the pupil was nearly or quite covered,[10] a more pronounced darkening resulted.

I am also in agreement with von Frisch in finding that covering both eyes completely with opaque screens resulted in a decided, though not maximal, darkening of the fish. I have records of 11 cases. Like von Frisch, I found considerable variability in the outcome of these experiments, even in the later ones (6 cases) in which care was taken that the blinders were completely impervious to light.[11] Von Frisch conjectures that, even when the eye is completely covered, the retina may be stimulated by light entering through the translucent tissues of the head. For this I find strong evidence. In a number of cases, fishes with $\frac{Bk}{Bk}$ eye-caps, including some of known opacity, were found to undergo unmistakable changes of shade when transferred from white to black containers and vice-versa. The changes were in the same direction as those displayed by normal fishes, though much less in degree.

In some other respects, on the contrary, the results of my experiments upon *Fundulus* are in sharp disagreement with those of von Frisch upon the trout. Covering one eye completely led to a less pronounced effect than covering both eyes. This experiment was performed deliberately four times, and came about unintentionally in a number of other cases through the accidental detachment of one blinder. Throughout the entire course of these experiments, indeed, the loss of one of the "corneas" was a frequent occurrence. In general, it may be said that the covering of one eye by any type of cornea led to a result intermediate between that expected from the given type of ex-

[10] I.e., hidden from view. In reality, considerable light must have entered from above.

[11] So far as revealed by a hand lens.

periment when both eyes were covered and that shown by the control fish upon the background in question. Frequently, indeed, the fish with one eye covered was almost indistinguishable from a control. Never was it recorded as displaying a more extreme condition than did the same fish when both eyes were covered.

I have likewise failed to note any trace of asymmetry in the chromatophore reactions of fishes, in consequence of covering a single eye. This was watched for closely during the later stages of the experiments.

Von Frisch obtained no results of significance from experiments in which the upper half of each eye was covered. He reports only three such cases, and apparently in each instance the fish was returned to a white background and not tested upon any other. This last circumstance, I believe, was responsible for von Frisch's failure to observe a highly significant phenomenon.

The $\frac{Bk}{W}$ experiment was performed by me 34 times, including only those cases in which the cornea was divided about equally between the opaque and the transparent areas. Of these, more than 25 yielded significant results, the rest being inconclusive, owing to complications of an irrelevant nature.

So long as they were kept in white containers, these fishes, in most cases, resembled the controls rather closely, or they were sometimes slightly darker than these. When transferred to a dark neutral gray,[12] on the other hand, they commonly remained distinctly paler than the background, and paler accordingly than control specimens, which usually conform themselves rather closely to backgrounds of this shade (Fig. 2).

In cases in which the darkened area of the cornea just reached the pupil, a paling effect ensued which, however, was less marked, in two fishes out of three, than when the dark and clear areas were of equal extent. In three cases, on the contrary, in which the darkened area nearly or quite covered the pupil, the fishes were distinctly darker than when this was of smaller extent.

Since these results with $\frac{Bk}{W}$ corneas are perhaps the most significant ones reported in this communication, they deserve some further discussion. Taken at face value, they seem to furnish strong support for the hypothesis that the chromatophore reactions of fishes are determined by the relative luminosity of the lower and upper portions of the visual field, the former stimulating the chromatophores in a positive sense (i.e., in the direction of conformity), the latter in a negative sense. And de-

[12] This was closely similar to the "slate color" of Ridgway's "Color Standards and Nomenclature."

spite certain complicating circumstances, I believe that this is, in reality, the most probable interpretation of the results.

Unfortunately for the solution of this problem, however, the *lower field: upper field* ratio is only one of several factors which influence the shade assumed by these fishes during an experiment, and it is not easy to disentangle the part played by these various factors. If this ratio

FIG. 2. Fishes wearing $\frac{W}{Bk}$ and $\frac{Bk}{W}$ corneas, along with a control. The first will be recognized as the darkest, the second as the palest, the control being intermediate. For photographic reasons, the fishes all appear somewhat too dark, relative to the background.

alone determined the result, one might expect that a fish, swimming over a well-lighted gray background, with total darkness everywhere above the horizon, should assume a maximum degree of pallor. In reality, the fish seldom does this. It is not unlikely, however, that this difficulty may be only an apparent one. The probability has already been pointed out that considerable light reaches the retina through va-

rious tissues of the head, beyond the margins of the false cornea. It is thus probable that the upper half of the visual field is not totally dark in these experiments.[13]

Again, it is known that the absolute degree of illumination has a decided influence upon the shade assumed by many fishes, irrespective of the background. This can be observed, in the case of *Fundulus*, by watching them as they swim in and out of shaded places. The $\frac{Bk}{W}$ specimens in the foregoing experiments exhibited this phenomenon in a high degree. They frequently assumed a grayish appearance while on the shaded side of the jar, becoming intensely pale upon emerging into the light.

A more serious complication, in our endeavor to interpret these experiments, arises from the unavoidable presence of stimuli due to the mechanical pressure of the eye-caps themselves, as well as to the suppression of all movements of the eyes. To test the effects of these stimuli, a considerable number of fishes (15) were fitted with "corneas" of clear, uncolored celloidin. The majority of these specimens became recognizably different in appearance from controls, the difference commonly lying in the direction of increased pallor, when on gray. In one of the cases, indeed, it is recorded that such a fish was even paler than the $\frac{Bk}{W}$ specimens which were available at the time for comparison.[14]

This circumstance doubtless weakens our argument based upon the effect of the $\frac{Bk}{W}$ corneas. Mechanical pressure upon the eyes tends to cause contraction of the melanosomes of the body. Some stimuli (e.g., darkening of the lower part of the visual field) are sufficient to overcome this effect. Where there are no opposing stimuli, it persists. May not this be the entire explanation?

That this explanation is not a satisfactory one is indicated by the fact that these cases of extreme paling were seldom met with among

[13] Unpublished experiments upon *Lebistes* have shown that both eyes of a young specimen may be extensively damaged by electrocautery without seriously interfering with the capacity of the fish to adjust itself to black and white backgrounds. In many cases, the eyes were so badly damaged that a person thus affected would doubtless rate as "totally blind." When complete destruction of the eye was accomplished, on the other hand, the capacity for response to background was entirely abolished.

[14] Leaving out of consideration another case, in which the available $\frac{Bk}{W}$ fishes had corneas 1/3 and 2/3 black, respectively, and remained relatively dark (see below).

fishes with transparent colorless corneas ($\frac{W}{W}$), while they occurred not only in a large proportion of the $\frac{Bk}{W}$ fishes, but in some of those in which the upper halves of the corneas were merely colored deeply without being rendered opaque. In eight out of nine experiments in which direct comparisons are possible with $\frac{Bk}{W}$ and $\frac{Bl}{W}$ specimens, including those most recently and carefully performed, the latter are recorded as being distinctly paler than the accompanying $\frac{W}{W}$ ones.

While von Frisch detected no effect from darkening the upper half of the field of vision, he does not contend that this part of the field plays no rôle whatever in determining the chromatic reactions of the fish to optic stimuli. From the fact that a more extreme darkening is produced by covering the lower half of each eye than by covering both eyes completely, he concludes that the former response involves a contrast effect, the contrasted elements being, of course, the upper and lower halves of the field of vision. Similarly, the results which he reports from covering one eye of a fish, namely, a more extreme darkening than when both eyes were covered, were held to substantiate the same view. The receptor elements here concerned, if I understand him correctly, were the *lower* half of the covered eye (the upper half being irrelevant), and the *upper* half of the uncovered eye (the lower half being irrelevant).

Bauer (1905) had earlier adopted a similar contrast theory in interpreting the results of his experiments with isopod crustacea. If one half of the visual field was darkened while the other remained illuminated, the animal became intensely dark, and it mattered not, in these experiments, which half was darkened and which was illuminated. Von Frisch believes that the fish differs from the isopod, in that the visual field of the former is, in a sense, polarized, the upper and lower halves playing different rôles.

As stated above, my experiments with *Fundulus* furnish conclusive evidence, for this fish at least, that an opaque covering over one eye results in a darkening which is not only far from maximal, but considerably less pronounced than that which results from covering both eyes.

Furthermore, results from the limited number of experiments in which the light and dark areas were arranged as shown in Fig. 1, *a* and *b*, were not in harmony with the contrast theory, at least in the form in which it is stated by von Frisch. When the field of vision was thus divided by a vertical line into anterior and posterior halves, the result

was in each case a slight or moderate degree of darkening. This was irrespective of whether the darkened half was anterior or posterior. The fish upon white was in some cases nearly as pale as the control, never was it of maximum darkness.

It is desirable at this point to include the results from the use of the four other types of cornea shown in Fig. 1, although their meaning is not clear. Two trials were made with each of these. Arranged in order of increasing darkness, the fishes, in both trials, lined up as follows on gray backgrounds: *e, c, f, d*. The first of these (*e*), in each case, was of a pallor approaching that of the $\frac{Bk}{W}$ fishes; the last (*d*) was of more than medium darkness. The data are insufficient for an interpretation of these results. As indicating that the lower half of the visual field exerts an influence in a positive direction upon the shade assumed by the fish, these results may be regarded as being in harmony with ones previously described. It will be noted that *d* and *f* were darker than *c* and *e*. On the contrary, the parts played here by the central and upper portions of the field are far from clear. Before reaching any safe conclusions, more numerous experiments would be necessary, in which the exact position of the pupils in relation to the clear areas was carefully controlled.

While I believe that von Frisch is correct in his contention that the upper and lower halves of the visual field play different rôles in relation to the pigmentary responses of fishes, I believe that he is wrong in restricting the rôle of the upper half to that of intensifying by contrast, when bright, the effect of a darkened lower half. My evidence as a whole seems to show that a darkened upper half has a definite negative effect in causing the fish to react to a background of medium or darker shade as if this were very much paler than it is in reality. This effect is complicated, it is true, by the influence of other stimuli, so that it is not always clearly recognizable.

If the upper and lower portions of the retina really have different potencies in determining the color reactions of fishes, this fact might be expected to reveal itself if, in any way, these areas could be transposed and the visual field thus inverted. As a matter of fact, I virtually accomplished this inversion in the case of several specimens. The fish was anesthetized and each eye severed from its connections with the orbit, save for the optic nerve. The ball of the eye was then rotated on its major axis through an angle of 180°, and fastened in its new position by stitches or otherwise. But the operation proved to be too drastic, and none of the fishes were thereafter capable of making any chromatic responses whatever, although four specimens lived for some

hours. Possibly a more skillful ophthalmic surgeon than I might have had better success.

An apparatus was also contrived, by which light could be projected into the eyes of fishes, held in position in special glass tubes made for the purpose. Small glass windows applied closely to the fronts of the eyes at the ends of brass tubes were so painted as to control the portions of the retina which were darkened or illuminated. Light was supplied by two electric flash-light lamps. Thus far, the results have not been very instructive, since the desired experimental conditions do not seem to have been fully realized.

In conclusion, I shall make rather brief mention of the experiments with colored corneas. No extended discussion of these experiments seems warranted, despite the very large amount of time which was devoted to them. This is owing to the inconclusive nature of many of the results. In my earlier experiments (January, February, and March, 1931) I seemed to find unquestionable evidence for a specific effect of yellow "goggles" upon the color of the fishes. In a considerable number of cases, a rather rich yellow resulted from this treatment, much as it did from keeping a fish for some hours or days upon a yellow background. Examples of such an effect were shown at various times to members of the Scripps Institution staff, as well as to visitors, and records of these experiments make it evident that this result was regarded at that time as the rule.

This marked yellowing of the fishes, due doubtless to the contraction of the melanosomes and simultaneous expansion of the xanthosomes, followed the insertion of $\frac{Y}{Y}$ corneas, as well as of $\frac{Bk}{Y}$, $\frac{W}{Y}$, and $\frac{Bl}{Y}$ ones. The effect would thus seem to be the result of stimulating the whole or a part of the retina by light of certain wave lengths. No contrast between different parts of the visual field was required. This is, of course, in harmony with the findings of von Frisch and others in experiments with the effects of monochromatic light.

Red likewise appeared to bring about a characteristic chomatic response, the fish becoming not intense red, but pale reddish brown, of the same shade as is assumed by this species when kept in a red container. Green and blue brought about no probable specific color changes, though blue corneas induced a neutral or sometimes slightly bluish gray, which differed distinctly from the buff or yellow appearance of fishes exposed to yellow.

In harmony with the previously described experiments, in which the upper or lower portions of the corneas were rendered entirely opaque

by black ink, those which were colored above or below exerted, in many cases, an influence upon shade, whether or not a specific color effect was shown. Thus, $\frac{Bl}{W}$ (blue over white) sometimes led to a condition paler than controls; $\frac{W}{Bl}$ to a condition darker than these.

In view of the apparently convincing nature of some of the foregoing experiments with yellow or partially yellow corneas, it was decidedly disconcerting, in a recent rather prolonged series of experiments, to encounter an almost complete absence of such effects. In order to determine whether these negative results were due to a change in the reactions of the fishes to color stimuli from any source, I next subjected a considerable number of fishes from the same stock, and of both sexes and various sizes, to the influence of yellow aquaria. Little or no specific color effect was now manifest. This was surprizing, in view of the striking responses to yellow backgrounds which had been manifested some months earlier,[15] and which were made the basis of experiments upon the possible effects of visual stimuli on xanthophyll formation (Sumner and Fox, 1933).

It is probably significant, in relation to these contradictory findings, that the first three of my series of experiments were conducted during the months of January, February, and March, while the last was carried on in late May and early June.[16] Some unknown metabolic change, due to season, must be credited with this undoubted difference in the chromatic reactions of these fishes.

Before closing, it should be stated that we unduly simplify the reactions of these fishes when we speak merely of their turning "pale" or "dark." The melanophores at different depths of the tissues respond more or less independently of one another. A fish may show little melanin in the superficial melanophores and yet remain fairly dark. When this deeper pigment disappears from view a translucent condition ensues. Likewise, the xanthopores react independently of the melanophores, and differences of color as well as of shade appear which frequently have nothing to do with the color of the background. The same fish, in its deeper tissues, may appear yellowish, greenish, or bluish, or may have a curious coppery tint, all without known causes. These changes naturally complicate any interpretations of experimental results. Anesthetization of the fish with urethane results not only in a marked

[15] Various students of chromatophores, e.g., Fries (1931) have likewise noted the marked effects of yellow backgrounds upon *Fundulus heteroclitus*.

[16] I have records showing that *Fundulus* gave pronounced responses to yellow-walled containers during every month from October to February, inclusive (October 23–March 1).

darkening, due to the expansion of the melanosomes, but commonly leads to a conspicuous display of the yellow pigment as well. These effects frequently do not disappear for a half hour, or even considerably more, after apparent recovery of the animal from the anesthetic, a fact which must be borne in mind by the observer.

Summary

(1) Darkening the lower half of the field of vision by the insertion of false corneas of celloidin resulted in a pronounced darkening of the entire dorsal surface of the fish (*Fundulus parvipinnis*), even when the animal was kept upon a white background.

(2) Darkening of the upper half resulted in no visible change (unless a slight darkening) when the fish was on white, but a fish so treated was commonly considerably paler than a control when placed upon a dark gray background. This fact, along with that stated in the preceding paragraph, seems to support the view that the shade which a fish assumes upon a given background is determined by the relative luminosity of the upper and lower portions of the visual field, the latter acting in a positive sense, the former in a negative.

(3) Various other stimuli, optical and otherwise, were found to affect the shade assumed by the fish, and these greatly complicated the interpretation of the experiments.

(4) Covering both eyes with opaque "blinders" resulted in the assumption of an intermediate shade. Contrary to the findings of von Frisch with the trout, the covering of one eye, in a fish kept on a white background, resulted in only a slight darkening. Furthermore, no trace of asymmetrical chromatophore response on the body was noted, following asymmetrical optical stimulation.

(5) In experiments during the winter and early spring, it was found that transparent "corneas" which were stained yellow, either throughout their entire extent, or in the lower half only, led to the assumption of a distinctly yellowish hue by the fish. In another series, some months later in the year, little evidence of such color changes was found. This accorded with the fact that these fishes responded markedly to yellow backgrounds during the earlier months, and ceased to do so during the later ones. Some physiological change of a seasonal nature appears to be involved here.

(6) Besides yellow, and to a lesser degree red, no other color evoked any specific color responses on the part of the fish. On the contrary, various color changes were manifest which obviously bore no relation to the chromatic stimuli.

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SEXUAL PHASES IN TEREDO

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The great disparity in the sex ratios reported for various species of shipworms indicates that the sex-differentiating mechanism must function somewhat differently than it does in animals that exhibit an equality or near equality of the two sexes. If examined during the breeding season the adults appear to be of separate sexes and frequently the females are many times more numerous than the males. More than eighty years ago Quatrefages reported a ratio of only five or six males in each hundred individuals of *Teredo norvegica*. Other investigators have claimed that various species of the genus are hermaphroditic. In Barnegat Bay, New Jersey, Nelson (1922) estimated that there was only one male to about every five hundred females of *Teredo navalis*. Similar statements appear in many treatises and textbooks. Grave (1928) found a much smaller excess of females at Woods Hole, Massachusetts, but May (1929) discovered only a single male among the individuals of *T. navalis* which he examined from the coast of Germany.

It has often been observed, however, that the mean size of the females considerably exceeds that of the males and it has been suggested that this disparity in size may be due to sex reversal of protandric females. On the other hand, the observed size differences might be assumed to result from a different metabolic rate in the two sexes, or perhaps the sex is controlled by the physiological conditions at the critical time of sexual differentiation. Only a few direct observations have been made to determine which, if any, of these alternative possibilities may be responsible. Yonge (1926) reports the examination of between two and three hundred adults of *T. norvegica* in summer at Plymouth, England, but found only separate sexes. At the same locality in February he found two hermaphrodites among many individuals of separate sexes and concluded that these two were in the act of changing from male to female. This was considered adequate evidence of protandry. Sigerfoos (1908) found many hermaphrodites among young individuals of *Bankia gouldi* and suspected that protandry is normal in that species of shipworm. Kofoid and Miller (1927) observed that males of *T. navalis* (*T. beachi* Bartsch) in San Francisco

Bay are commonly smaller than females, but found no evidence that an actual change of sexual phase might occur.

It is obvious that the question can be settled only by following the growth stages during the entire life and under a variety of environmental conditions. Such is the object of the present investigation.

In a recent publication (Coe, 1933), reporting the destruction of mooring ropes in Long Island Sound by *Teredo navalis morsei* (*T. morsei* Bartsch, 1922), mention was made of the evidence for protandry in that species. Rope is such an unnatural and unsuitable material for the sustenance of shipworms that normal growth is inhibited and only dwarf, or stenomorphic animals are found. Yet such individuals may reach sexual maturity and produce a relatively small number of normal

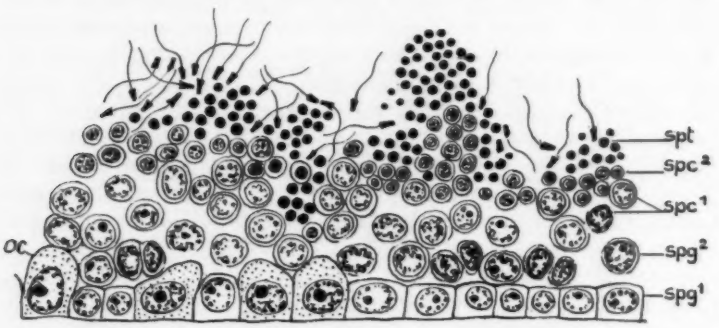

FIG. 1. Gonad of initial male phase, male type, with a few spermatozoa already formed; cortical layer with only a few small differentiated ovocytes (*oc*); *spg*¹, *spg*², primary and secondary spermatogonia; *spc*¹, *spc*², primary and secondary spermatocytes; *spt*, spermatids.

gametes. All young individuals appear to pass through a functional male phase and most of them later become females.

Histological study shows that the developing gonad is always more or less distinctly bisexual, each follicle consisting of an outer or cortical layer of ovocytes with a mass of spermatogenic cells filling the lumen (Figs. 1–3). Proliferation of spermatogonia proceeds rapidly and spermatogenesis follows so quickly that the gonad may be filled with ripe spermatozoa within five to six weeks after the young animal has completed metamorphosis. Before the spermatozoa have been discharged, however, the ovocytes in the cortical layer have begun the deposit of yolk in anticipation of the following female phase (Fig. 3). Entirely parallel conditions are found in *T. navalis novangliæ* at Woods Hole (Figs. 4–6).

In both these varieties there is much variation in the relative pro-

portion of cortical ovocytes and medullary spermatic tissue, with a fairly definite correlation between the early deposition of yolk and the abundance of male cells. With the exception of older animals which had

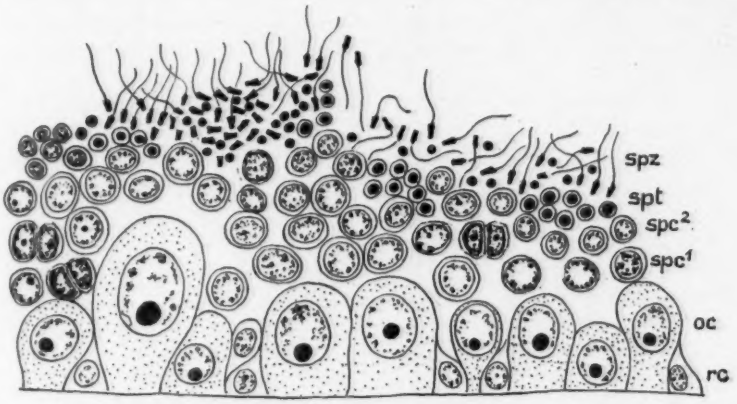

FIG. 2. Primary gonad of protandric female, with cortical layer of yolk-forming ovocytes and numerous ripe spermatozoa (*spz*) in the lumen of the follicle; *rc*, residual cells (mainly smaller ovocytes) which form the germinal cells of the gonad after the first ovulation; other letters as in Fig. 1.

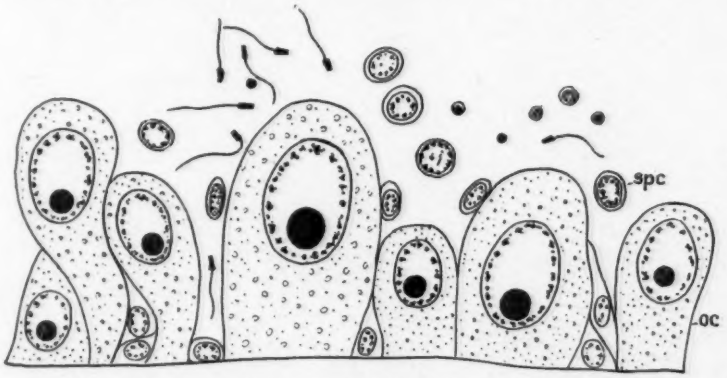

FIG. 3. Later stage in the gonad of protandric female preceding the first spawning period, showing the cortical layer of large ovocytes (*oc*) and small residual cells, with a few spermatocytes (*spc*), spermatids, and spermatozoa from the initial male phase remaining in the lumen.

previously completed the male phase and showed only female characteristics, both types of sexual cells were present in nearly every one of more than two hundred gonads cut in serial sections. The youngest

sexually mature individuals would naturally be classed as males and the oldest as females, with an intergrading series between.

The great variation in the proportion of cells characteristic of the two sexes that is also found in animals of apparently the same age indicates either an environmental influence or a dominating action of the genetic factors of one sex or the other. In general, the growth

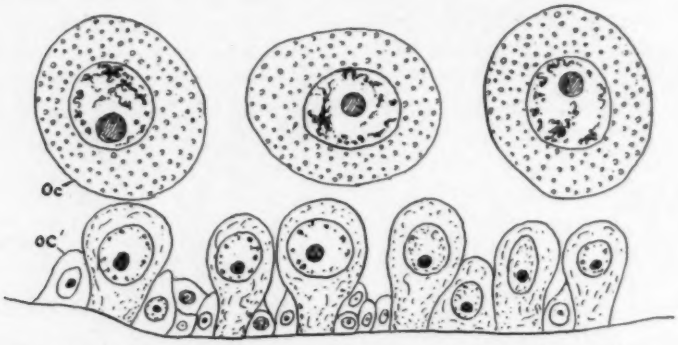

FIG. 4. Ovary at time of first ovulation, showing half-grown residual ovocytes (oc^1) and small indifferent cells remaining after the discharge of the ripe ova (oc).

FIG. 5. Initial male phase of gonad of protandric female with large ovocytes (oc) which reach maturity as soon as spermatogenesis has been completed. Smaller ovocytes (oc^1) form the ova of the second ovulation. Other letters as in Fig. 1.

of the cortical ovocytes seems to inhibit to some extent the proliferation of the spermatogenic cells, possibly through the assimilation of all the available nutriment. Thus the gonads of some individuals at the time when the first spermatozoa are formed may have a characteristically male appearance, with only a few scattered ovocytes of small size (Plate I, Fig. 8) or they may more closely resemble ovaries, each follicle having

a complete cortical layer of large ovocytes enclosing relatively few spermatogenic cells in the lumen (Plate I, Fig. 7; Plate II, Fig. 10). Not infrequently a more definitely hermaphroditic condition exists (Plate II, Figs. 11, 12) or various parts of the same gonad may differ in these respects (Fig. 6).

In addition to the protandric females which under favorable conditions constitute the vast majority of the population, there occur a few individuals which appear to retain the male phase through life.

These may be called true males to distinguish them from the male phase of protandric females. Most of these true males remain much smaller than the females of the same age but some of them may be as large as the average female. Selection for size alone would thus segregate most, but not all, of the males in a group of uniform age. The proportion of such males in a population seems to be greatest where the conditions for rapid growth are least favorable, indicating,

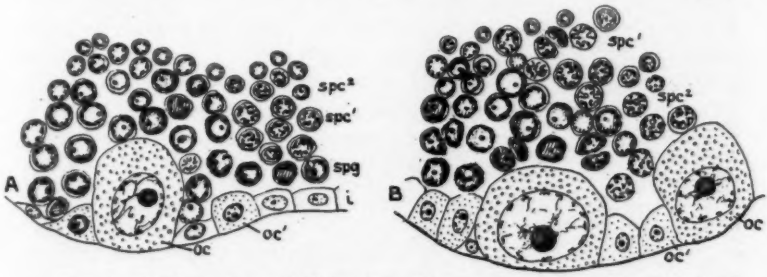

FIG. 6. Portions of two follicles from the same gonad, showing variation in the relative proportions of ovocytes (*oc*, *oc*¹) and spermatogenic cells; *i*, indifferent residual cells. Other letters as in Fig. 1.

perhaps, that the environment may influence the expression of the sex-differentiating mechanism in the teredo as it appears to do in the oysters (Coe, 1932*a*, and *b*).

It has been repeatedly observed that after the animal reaches the female phase the first ovulation may take place while the body is relatively small. Only a few hundred ova are then produced, although the later crops may each include more than a hundred thousand.

Several crops of ova may ripen in each well-nourished animal during the breeding season which extends over a period of about five months. As the size of the body increases the number of ova at each ovulation tends to become greater.

In this species the ripe ova pass from the genital duct to the mantle cavity, where they are fertilized by spermatozoa brought into the body with the water which passes through the gills. During early cleavage the eggs reach the gill chambers and in this situation the embryos develop

into larvæ which are later liberated into the water as free-swimming veligers (Nelson, 1922; Kofoid and Miller, 1927; Grave, 1928). Several thousands of these bivalve larvæ may be discharged from the body within the space of a few minutes.

The eggs usually, but not always, leave the ovary before the disappearance of the germinal vesicles. Contact with the sea water in the mantle cavity results in the rapid dissolution of the nuclear membrane and formation of the first polar spindle, if this has not already been formed in the ovary. The entrance of the spermatozoön leads to the formation of both polocytes. In contact with the gills the eggs complete cleavage, after which the young embryos become closely packed between the gill plates, where they continue development for two to three weeks.

After ovulation the follicles of the ovary collapse to some extent but large numbers of residual ova remain to form the basis of the second crop of ova (Fig. 4). A second ovulation commonly follows shortly after the first crop of veligers has left the branchial chambers or about three weeks after the first. Occasionally, however, particularly in the early part of the breeding season, the second crop of ova reaches the gills while many of the larvæ of the first crop are still present. It is estimated that the larvæ spend from two to three weeks in the free-swimming stage before settling upon the wood in which they metamorphose to the adult form. While the successive crops of young are being produced during the summer the animal has been rapidly growing in size and elongating its burrow in the wood, provided the nutritive conditions remain favorable and the extent of the remaining wood permits.

The life span of the teredo is seldom more than one year, but the rate of growth is very rapid under favorable conditions. Sexual maturity may be reached within six weeks after metamorphosis in the warmer part of the year, when the body has reached a length of 15–30 mm. Growth continues, however, and an animal a year old may have

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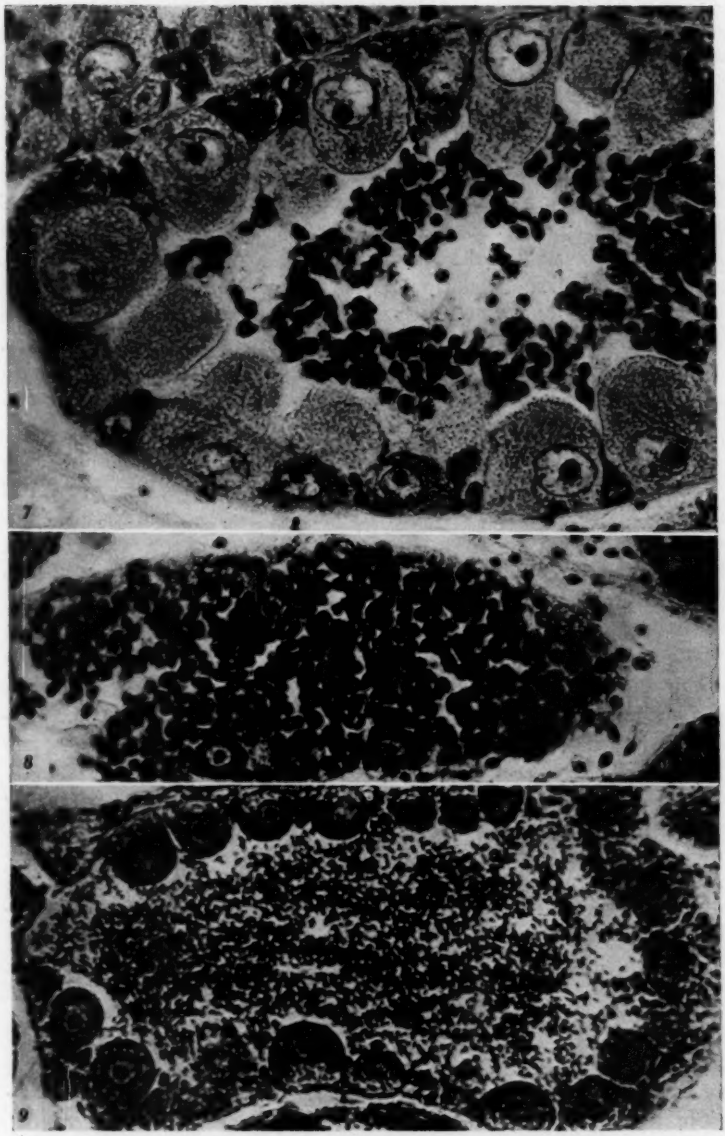
EXPLANATION OF PLATE I

FIG. 7. Primary gonad with strong female tendency, showing the cortical layer of large ovocytes, with a relatively small number of spermatogenic cells in the lumen.

FIG. 8. Primary gonad with strong male tendency, with only a few differentiated small ovocytes in cortical layer and abundant spermatogenic cells in the lumen.

FIG. 9. Initial male phase nearly completed; lumen of follicle filled with vast numbers of spermatozoa; cortical layer with half-grown ovocytes. Transition stage from male to female phase.

PLATE I



attained the great size of 40 cm. in length and more than 9 mm. in diameter (Grave, 1928). Usually, however, the limits of the available wood do not permit so extensive a growth. All observers agree that relatively few sexually mature individuals survive their first winter and that death normally occurs before the end of the second winter in these localities, except in the case of those that were very immature during their first winter.

Several broods of young may have been produced in the meantime but there is no evidence of a second change of sex. Having reached the definitive female phase the individual evidently continues to function as that sex throughout the rest of its life.

The histological basis for the changes which the gonads undergo during the development of each of the sexual phases has been studied at all seasons of the year and under various environmental conditions. The principal features are reported on the following pages.

Histological Basis of the Sexual Phases

Both at Woods Hole and in Long Island Sound the breeding season of the respective varieties of *Teredo* extends over a period of several months. The first ovulation may begin early in May for some individuals and the last usually continues into October for others. In a large and well-nourished individual the aggregate of the young produced may exceed a million. Larvæ are present in the water at all times during the breeding season (Grave, 1928).

Consequently, as sexual maturity may occur in the young animals within six weeks after metamorphosis, there are innumerable overlapping broods in the population of a locality each season. The conditions are further complicated by the fact that the metamorphosing young are likely to settle upon the same timbers that are already occupied by earlier arrivals. Only by placing pieces of wood in the water at frequent intervals can the ages of the occupants be ascertained.

If such a wooden trap be set in the spring in an infested locality and examined the following spring, animals of many different ages

Explanation of Plate II

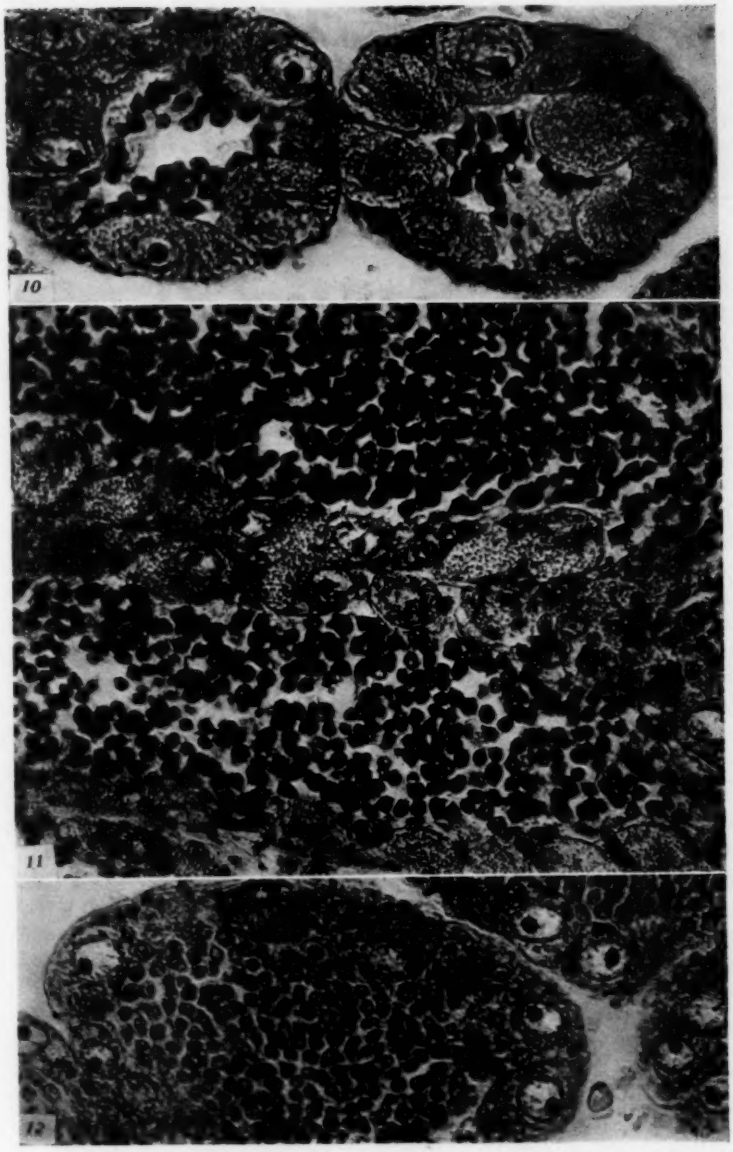
Sections of gonads of three individuals of *T. n. novangliæ* from Woods Hole, showing different aspects of sexuality in protandric females in early spring (March 29) and shortly before the onset of active spermatogenesis.

Fig. 10. Portion of gonad of feminine type of protandric female, with relatively few spermatogonia in lumens of follicles and large ovocytes on cortex.

Fig. 11. Portion of gonad of masculine type of protandric female, with numerous spermatogonia and spermatocytes in the lumens of the follicles. Ovocytes form complete cortical layer.

Fig. 12. Portion of gonad of the hermaphroditic type of protandric female, with only a few large ovocytes and numerous spermatogonia.

PLATE II



and of different aspects of sexuality will probably be obtained. The large ones will show a preponderance of females while the smallest, if sexually differentiated, will be exclusively in the male phase unless the block is so greatly overcrowded that the differentiated females have had no space for growth. In such a locality, if unprotected piling or other wooden structures have been in the water for a year or more, eight or nine somewhat different phases of the life cycle of the teredo, all of which are sometimes found in a single piece of timber, may be present at the beginning of the breeding season: (1) Large adult females, nearly one year old, which completed the protandric male phase early the preceding summer. These are not usually very numerous but each may contain an enormous number of ova. (2) True males, of the same age as the oldest females; very few but filled with large masses of spermatozoa. (3) Younger females which completed the male phase during the middle or later part of the preceding summer, also with very numerous ova. (4) Small females which reached the female phase at the end of the last breeding season. (5) True males which shed one crop of spermatozoa at the end of the last breeding season. (6) Young hermaphroditic males, filled with spermatozoa but also containing half-grown ova, showing that these individuals represent the initial male phase of the protandric females; these are usually far more numerous than all the other types combined. (7) Young females in which the initial male phase may have been abortive. (8) Young true males, differentiated from the hermaphroditic males by the presence of only a few small ovocytes on the walls of follicles containing an unusually large number of spermatogenic cells. (9) Very small individuals which completed metamorphosis late in the autumn and have not reached sexual maturity; these may remain so small as to escape notice during the winter but their presence becomes evident in the spring. Not all of the phases enumerated can be easily distinguished from each other, for there are many intergrading stages associated with each phase (Plates I and II, Figs. 7–12) and the rate of growth is highly variable.

If the timber has been in the water only since the middle of the preceding summer the oldest groups will not be found, and if it was placed in the water toward the end of summer only the younger groups will be present.

Perhaps it may be assumed that in the teredo, as in other pelecypods, the necessary stimulus for ovulation is provided by the presence of spermatozoa of the same species after the water has reached the requisite temperature and that the spermatozoa are discharged by the sexually mature males through the stimulus of the rise of temperature alone. If such be the case there must occur a widely spread emission of sperm

by the true males and especially by the more numerous protandric males at the beginning of the breeding season as soon as the temperature of the water becomes sufficiently high. This occurs during the first half of May in the localities here considered. Grave (1928) records this critical temperature as 11°–12° at Woods Hole, although Nelson (1928) finds that the teredo in Barnegat Bay, New Jersey, requires a temperature of about 15° to call forth the spawning reaction. The latter has generally been considered the critical temperature for the so-called *T. navalis* over the greater part of its supposedly extended range both in the Atlantic and the Pacific.

The individuals which reached the female phase during the preceding summer and autumn will respond one after another as their ova become fully mature. Two to three weeks later the first larvæ are liberated into the water and after a free-swimming period of about the same length of time a new infestation of the wooden structures in the vicinity begins.

As soon as the sperm of the much more numerous young protandric individuals are shed, their ova ripen and more abundant crops of larvæ are set free in the water day by day as the breeding season advances. A rapid rise in the temperature early in the breeding season may thus be followed by swarms of larvæ. A second period of excessive abundance is likely to occur shortly after these larvæ have reached the female phase of sexual maturity eight to ten weeks later. In the meantime the older females continue the supply of newly hatched larvæ daily. It has been noticed frequently that the teredo will mature sexually and produce a small number of gametes even if the wood is so overcrowded as to prevent the normal growth of the individual. Even under so unfavorable an environment as rope, when the entire population consists of dwarfs, sexual differentiation seems to follow its normal course and the protandric females produce a small number of ova after completing the functional male phase (Coe, 1933).

Although the expression of femaleness usually increases with advancing age, this rule appears to have some exceptions, for an occasional animal is found in which the hermaphroditic male phase is accompanied by the degeneration of ovocytes previously formed. It seems not improbable that such degeneration may occasionally include all the ovocytes and if such is the case a true male phase might follow a bisexual phase in which many ovocytes had already begun the deposit of yolk. It is obvious that the gonad of the young animal is not an infallible index of the potential sexuality of the fully mature individual.

While all grades of bisexuality are found in the gonads, self-fertilization is not usual, for the sequence of male and female phases is so regulated that most of the spermatozoa of the initial male phase

are discharged before the ova are ready for fertilization. Normally an interval of at least several days intervenes between the two sexual phases. Sometimes a small number of ripe ova may be formed before spermatogenesis is completed, but no case has yet been observed in which the gills contained embryos previous to the discharge of all the sperm.

Development of Gonad

In its early development, the gonad of *Teredo* is not greatly different from that described for other pelecypods (Coe, 1932*a*, 1932*b*), with a branching system of tubular lobes or follicles, each of which is provided with a cortical layer of gonia, while the lumen is filled with proliferating spermatogenic cells (Fig. 1).

The cortical layer, which is usually well demarcated in later stages of development, remains after the spermatogenesis of the preliminary male phase is completed to form the residual cells. The latter, as stated above, have already become differentiated as ovocytes in the protandric females (Figs. 2, 3), but most of them furnish spermatogenic cells in the few true males that accompany their numerous sisters.

Protandric Females

In the early part of the preliminary male phase it appears to be impossible to distinguish sharply between the protandric females and the true males and in both types some of the cells of the cortical layer become differentiated as ovocytes (Plates I and II, Figs. 7–12). In the former the proportion of ovocytes is greater and they increase in size earlier, while in the true males the ovocytes remain small and the spermatogenic cells in the lumens proliferate rapidly. As spermatogenesis continues and the number of spermatozoa increases, additional ovocytes grow in size and begin the deposition of yolk (Fig. 2). With the completion of spermatogenesis the ovocytes are usually about half grown (Plate I, Fig. 9) and after the discharge of the spermatozoa the animal has all the characteristics of a female. Not infrequently some spermatozoa are retained during the early female phase (Fig. 3), and it is not improbable that such may be present when the first ova are released into the mantle cavity. Self-fertilization might then be possible, although normally the eggs are fertilized by sperm from other individuals. The fertilized eggs, as already stated, are retained in the gill chambers until the larval stage is well advanced.

The male phase may be passed so quickly that larvæ metamorphosing in June have reached the female phase and produced a new brood of larvæ by the middle of August. In this brief period of eight to nine

weeks the body may attain a length of 80 mm. or more and a diameter of 7 mm. under favorable conditions.

In young animals which complete the initial male phase in the autumn the female phase will be developed during the winter and retained the following summer. It is thought that few if any individuals survive two winters after sexual maturity and there is no evidence that a second sex reversal may ever occur after spawning has been completed at the end of the first breeding season.

There is often a fairly close correlation between the stage of spermatogenesis in the protandric females and the growth of ovocytes in the cortical layer of cells. In young animals during the summer at least a few spermatozoa may be formed before the deposition of yolk commences in most of the ovocytes. But if the gonad develops in the late autumn spermatogenesis is inhibited and the ovocytes continue their growth during the winter and early spring. The large ovocytes then form a complete cortical layer, pushing the spermatogenic cells into the lumens of the follicles (Fig. 5; Plate I, Fig. 7; Plate II, Fig. 10).

In these females there is always more or less degeneration among the spermatogonia, apparently due to the absorption of all the available nutritive materials by the rapidly growing ovocytes. In some cases the entire mass of spermatogenic cells seems to degenerate, so that the preliminary male phase may be abortive, and it is not improbable that an occasional individual may function as a female at its first reproductive period. The conditions in such cases are somewhat similar to those described for the American oyster, where an abortive male phase precedes the functional female phase in all individuals which spawn as females in their first breeding season (Coe, 1932*b*).

True Males

In the second type of gonad the preliminary male phase is of longer duration and the crop of spermatozoa is much larger. In these individuals most of the residual cells appear to be potentially spermatogonia, indicating that the succeeding sexual phase will likewise be male. A few small ovocytes are invariably present, however, (Plate I, Fig. 8) and it is not improbable that a later sex reversal may sometimes occur.

Serial sections of the gonads of a group of functional males show that all phases of bisexuality occur. These lead in a graded series from true males to weakly feminine and strongly feminine hermaphroditic male and protandric female phases. A single collection which was made early in the breeding season (June 24–28) at Woods Hole will serve as an example. Of the 116 individuals taken from piles, 46 were found to be in the functional male phases, 47 were ripe females, and

23 were females bearing embryos. The sex ratio was thus 66 functional males to 100 females. Sections were made of the gonads of 36 of the 46 males; 7 were obviously hermaphrodites, containing ripe sperm and nearly mature ova; 17 were strongly feminine hermaphroditic males, with half-grown ovocytes; 4 males had smaller and fewer ovocytes (weakly feminine); while only 8, or about 26 per cent of the animals with ripe sperm could be classed as true males. Most of the others presumably represent transition stages in the development of the definitive female phase of sexuality. Another collection contained 13 true males, or 30 per cent of the 43 functional males of which the gonads were sectioned. Both were in agreement as indicating a ratio of 10 to 11 true males to 100 functional females in the early part of the breeding season.

Since most or all of the hermaphroditic males would have reached the female phase by midsummer, the population, if no new young had been added and there had been no differential mortality, would presumably have shown at that time only 8.6[1] males to 100 females, a proportion close to that which Quatrefages reported for *T. norvegica* in 1849. The proportion would obviously be changed by later reversals in either direction of the sex tendency indicated by the gonads at the time of examination.

The true males develop more slowly than the other sexual types and consequently their sperm are available for the fertilization of the first crop of ova produced by their more rapidly maturing protandric sisters.

Hermaphroditic Males

In addition to the obviously protandric females and the so-called true males many young individuals are found, both in the summer broods and in the overwintering young adults, which appear to be of an intermediate sexual nature. These are characterized by having only a few ovocytes in the cortical layer (Fig. 6; Plate II, Fig. 12). Such hermaphrodites may produce a small number of ripe ova immediately after spermatogenesis has been completed. They form a graded series in which the expression of the initial male phase has been less definite or the transition from male to female phase less precise than usual.

Under favorable conditions shipworms grow with great rapidity during the warmer portion of the year and the wood may be completely riddled by the borers before any of them have attained the female phase. Heavy infestation and crowded conditions may cause not only dwarfed individuals but not infrequently the early death of all.

[1] Actual numbers found August 18–21, 1933, were 18 males to 202 females—a surprisingly close approximation to the predicted ratio.

Change in Sex Ratios During the Breeding Season

Because of the protandric nature of the teredo there will obviously be a progressive change in the sex ratios of the population from maleness to femaleness as the breeding season advances. In a population of overwintering young adults the vast majority will be found to be in the initial male phase in late winter and early spring, while the proportions of the two sexes will be reversed after the male phase has been completed and the spawning period is well advanced.

An examination of the teredos in boards taken from a framework in the Eel Pond at Woods Hole May 19, 1933, showed 138 individuals in which active spermatogenesis was in progress and 30 which were classed as females because of the presence of ripe ova or of embryos in the gill chambers. Hence the ratio of functional males to females at this time was 460 males to 100 females (Table I). Other boards

Table I

Change in Sex Ratio during Breeding Season

Date	Male phase	Female	Total	Ratio, Male to Female
May 19	138	30	168	460 : 100
June 7–13	53	121	174	44 : 100
June 20	15	31*	46	48 : 100
June 16†	23	42	65	55 : 100
June 24–26†	46	70	116	66 : 100

* Most of the large females had died during the preceding week.
† From a different source.

from the same source examined when the first larvæ were ready for liberation (June 7–13) yielded 53 individuals in the male phase and 121 which contained ripe ova or had larvæ in the gill chambers. The sex ratio has now changed from 460 males to 44 males for each hundred females.

Histological study of the gonads of over two hundred individuals is in full accord with the observations on the living animals. Most of the functional males actually represent the male phase of protandric females as is proved from their gonads when cut in serial sections. Sections of 26 of the 138 individuals determined as functional males from a microscopic examination on May 19 showed that 21 of these were undoubtedly protandric females and only 5 were true males (Table II).

TABLE II

Sexual Phases Preceding First Ovulation

Date	Male phase	Male (Protandric Female)	Total Male	Female	Total
Mar. 29	4	18	22	0	22
Apr. 27	3	9	12	0	12
May 2	1	11	12	0	12
" 16	2	3	5	2	7
" 29	2	3	5	4	9
May 19*	5	21	26	20	46
Sept. 30†	41 (immature)	12	53	1	54

* Selected samples from a different source.

† Estimated age about five to six weeks after metamorphosis.

Since the transition stages from the male to the female phase form a graded series, it is obviously impossible to separate accurately the two sex groups. This is made even more difficult by the fact that a few spermatozoa may remain in the gonads in the early female phase long after spermatogenesis has ceased in most of the follicles, as well as by the presence of spermatozoa from other individuals which tend to accumulate in the mantle cavities of ripe females. As an arbitrary rule the animal has been classed as a male if any considerable number of spermatozoa remain in the gonads, even if there are fully grown ova also. The difference of a single day may thus change the classification of certain individuals.

Later in the breeding season (June 20), after the first crops of larvæ had been liberated, some animals could still be obtained from the same source but most of the large females in the original population had died in the meantime. In spite of this selective death rate, other individuals had reached the female phase to make the ratio of 48 males to 100 females. Instead of more than four functional males to each female, as was the case at the beginning of the breeding season, there was now less than one male to two females. Material from another locality was in close agreement (Table I). Grave (1928) found a ratio of 42.5 males to 100 females in July and early August.

On August 20, 1933, a collection of 220 of the largest, and evidently oldest, individuals from boards placed in the water June 10 contained 18 males, 63 transition females, 82 ripe females, and 57 females bearing larvæ. The sex ratio was therefore 9 males to 100 females. But from a lot of more than 500 smaller, and presumably younger, individuals all except about 2 per cent were functional males. About 38

per cent had reached the early transition stage. The 2 per cent were dwarfed females of the older age group.

A similar population at Woods Hole living in thin pieces of lath and consequently dwarfed in size showed a parallel condition. Previous to the breeding season (March 29), a study of the gonads of 22 of these indicated the bisexual nature of each individual, although there was a great range in the relative size and proportion of the cells characteristic of the two sexes (Plates I and II, Figs. 7, 8, 10–12).

Although spermatogenesis had not yet advanced further than the formation of spermatocytes, some of the gonads were distinctly of the female type, with a cortical layer of large ovocytes in each follicle and relatively few of the small cells, which later prove to be spermatogonia, in the lumens. Others were predominantly male in appearance, consisting of masses of spermatogonia with only a few differentiated ovocytes in the cortical layer (Fig. 5). Still more numerous were the intermediate phases which were typically bisexual and could not be classed as predominantly of either sex (Fig. 6), although they were potentially protandric females.

Table II shows the change in proportion of the sexual phases during the two following months as determined from serial sections of the gonads. All were young animals of the previous year and hatched too late to complete the initial male phase before winter. No functional females appeared before the middle of May, when spermatogenesis was completed in a few individuals. The proportion of females increased thereafter. Although the numbers are small, the data agree with other observations which indicate a regular increase in the proportion of adult individuals in the female phase as the season advances.

A piece of wood infested in early summer and examined in the winter or early spring will reveal an entirely different ratio of sexes. Many of the animals in such wood will have completed the initial male phase late in the preceding summer and will consequently appear as females during the winter. Usually, however, a larger number of smaller and younger males will be found in the same block. Such represent later settlers which have not yet completed the protandric phase. A very few large males, representing the true males of the older age group, may also be present.

It probably happens not infrequently that the young males in one timber furnish the spermatozoa which fertilize the ova of the older females in other timbers in the vicinity. The sperm taken in by the female evidently may be retained for some time in the mantle cavity, for in not a few cases swarms of spermatozoa were found in the mantle cavities of fully ripe females. It is conceivable that secretions, includ-

ing carbon dioxide, within the female's body may retard the movements of the spermatozoa until they are stimulated to more vigorous action by secretions from the ova as the latter come in contact with the sea water in the mantle cavity and their germinal vesicles break down. The eggs are fertilized almost immediately after reaching the mantle cavity. The oviduct is so widely open that water from the mantle cavity may reach the terminal chambers of the ovary and cause the formation of polar spindles in some of the ova before they leave the oviduct.

In the middle of summer the proportion of the sexes will be entirely different in wood that contains only the overwintered population than it is in pieces recently infested. Selection either by age or by size will then yield groups which are nearly all of one sexual phase or the other. This will account for the great disparity of the sexes noted so often by previous investigators.

In random selection from a general population there will be a great excess of males in the early part of the season, due, as stated above, to the predominance of young individuals in the initial male phase. The proportions will then change gradually to a similar excess of females, as soon as spermatogenesis is completed and the sexual phase changes. Later there will again be an excess of males as the new crop of young becomes sexually mature in July. A less marked influence on the sex ratios may possibly result from environmental conditions.

Conclusion

In comparing the sexual phases of the teredo with those of other animals and particularly with other pelecypods, there seems to be a closer similarity to those of various species of oysters than to any other forms. Protandry is of frequent occurrence in molluscs of widely divergent groups but in none of these is there such an enormous excess of females in the adult population as is here reported for the teredo.

In the teredo the great majority of individuals experience one change of sex. The genetic factors involved in the sex-determining mechanism appear to be so adjusted that the internal environment, not improbably of hormonal nature, normally stimulates the activity of the medullary cells of the follicles during immaturity, leading to the initial male phase of sexuality. Thereafter the cortical cells usually become activated and the animal functions as a female. The external environment may supplement the action of these genetic factors in determining the extent to which the male phase is expressed. This may lead to the observed graded series of hermaphroditic males.

There still remain for consideration the relatively few individuals which have been called true males because they retain the initial male phase for a much longer time than the others and perhaps throughout life. Similar males occur in both larviparous and oviparous oysters, as well as in other groups. It seems probable that they may be dependent upon particular combinations of genetic factors, influenced by physiological (metabolic) conditions during the period when the sexual phase becomes differentiated. The graded series of sexual forms leading from these true males through weakly to strongly feminine protandric females supports the well-known quantitative theories of sex as formulated by Goldschmidt and later by Castle. But it must be emphasized that the series is dependent not only upon the initial sex factors but also upon an age factor, since the female phase appears only after an initial male phase.

In the developing gonad there seems to be little, if any, antagonism between the germinal cells characteristic of the two sexes, for the ovocytes continue their growth, though less rapidly, while spermatogenesis is in progress. The observed sexual phases and the anomalous sex ratios can, perhaps, be best explained by assuming the potential bisexuality of all individuals, associated with alternating genetic factors which bring into activity first one phase of sexuality and then the other. In the California oyster the sequence continues year after year (Coe, 1932*a*), while in the American oyster, which sometimes lives to the age of ten years or more, a facultative change of sex may occur during the winter following a season of functional activity in either sexual phase (Coe, 1932*b*). In the teredo, however, death usually ensues after one full season of female sexuality.

The individuals of all species of *Teredo* live in crowded communities and the reproductive period extends over so long a season that the rapidly maturing young of the early broods may reach the functional male phase in time to fertilize the later crops of ova produced by their own mothers or by other individuals which have attained the female phase in the meantime. Such initial males, together with the very small proportion of true males usually present, afford ample provision for the fertilization of the half-million or more eggs which may be produced by each of the females which constitute the vast majority of the fully adult population. The extraordinary disproportion of the sexes is thereby compensated.

Summary

1. *Teredo navalis morsei* in New Haven Harbor and *T. n. novangliae* at Woods Hole are essentially protandric, nearly all females pass-

ing through a preliminary functional male phase before reaching the definitive phase of sexuality.

2. The primary gonad is bisexual in nature, having a cortical layer of potential ovogonia, with spermatogenic cells filling the lumen of each follicle.

3. The gonads of young individuals in early sexual maturity indicate three intergrading but more or less well demarcated types of functional males: (a) initial males, representing the protandric phase of the females; these have a complete cortical layer of ovocytes surrounding the relatively small amount of spermatogenic tissue in the lumens of the follicles; (b) hermaphroditic males; these have relatively few large ova scattered among the spermatogonia of the cortical layer; (c) true males; most of the ovocytes in the cortical layer remain small or degenerate.

3a. In the protandric females the cortical cells become differentiated into ovocytes and begin the deposition of yolk while spermatogenesis is in progress in the lumens. The functional male phase may be reached within six weeks after metamorphosis and is of brief duration. A few spermatozoa may be retained until the first ovulation, after which the animal seems to have exclusively female characteristics.

3b. In hermaphroditic males the functional male phase is of longer duration than in typical protandric females and the number of spermatozoa produced is much greater. A later transformation to the female phase is presumably of usual occurrence, but is not invariable.

3c. True males form a small proportion of the population. Growth continues during a long period of spermatogenesis. After the discharge of the first crop of spermatozoa most of the residual cells still retain their male characteristics and no evidence of a later change of sex has been found, although small ovocytes are usually scattered among the cortical cells.

4. The proportion of individuals in the two functional sexual phases changes with the season, there being a great excess of males at the beginning of the breeding season due to the protandric nature of the young animals in the early period of sexual maturity; there is a corresponding excess of females later in the season, due to the change of sexual phase after spermatogenesis in the initial male phase has been completed. After the end of July the proportion of males may again be greatly in excess if the new crop of young is included. Selection of the larger (and older) individuals will then yield mainly females, while the smaller (and younger) animals may all be in the male phase.

5. In the early part of the breeding season the populations from two localities each consisted of about 33 per cent functional males and 67

per cent mature females. Less than one-third of the functional males were classed as true males, the others indicating the potentiality of a later change to the female phase. By the middle of August only 9 males to 100 females were found in the older age group.

6. After the assumption of the definitive female phase there is no evidence of a second sex reversal.

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STUDIES ON AMPHIBIAN METAMORPHOSIS. XI. DEVELOPMENT AND REGRESSION OF DERMAL PLICÆ FOLLOWING HOMOPLASTIC AND HETEROPLASTIC TRANSPLANTATION

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INTRODUCTION [1]

The writer (1931) has previously pictured and described the normal histological developments of dermal plicæ structures as they occur during the metamorphosis of *Rana pipiens* larvæ. Reciprocal, autoplastic transplantations were also made between potential lateral and dorso-lateral dermal plicæ integument and integument of the back and belly. During involution it was observed that the back and belly integumentary transplants never developed dermal plicæ structure, although directly adjacent to normally developing dermal plicæ. The potential dermal plicæ integument, previously transplanted to the back or belly, however, always underwent a normal development resulting in fully-formed dermal plicæ by the close of larval involution. It was concluded, therefore, that certain areas of integument become specific for dermal plicæ development before the onset of larval involution, and that such development is the result solely of influences present in the blood-stream during metamorphosis. Apparently, the location of the potential dermal plicæ integument in no way influences its later histological developments.

As a result of the above described work, several interesting problems presented themselves which may be briefly stated in the following questions:

1. Is dermal plicæ development possible following heteroplastic transplantation?

2. Is the stimulus for dermal plicæ development still present following involution of the larva?

3. Can mature dermal plicæ integument maintain its histological structure when transplanted to a non-metamorphosing host or is its stability dependent on influences present only in the metamorphosing or fully-metamorphosed animal?

[1] Since no extended discussion concerning the various problems relating to amphibian dermal plicæ and integumentary glands is intended in the present paper, the reader is referred to the bibliographies of Dawson (1920), Helff (1931), and Weiss (1908), (1915).

The present paper concerns the results of transplantation experiments made in an attempt to answer the above questions. The following series of transplantations were made:

1. Reciprocal, heteroplastic transplantation of potential dermal plicæ integument between *R. catesbeiana* and *R. palustris* larvæ.
2. Reciprocal, heteroplastic transplantation of potential dermal plicæ integument between *R. catesbeiana* and *R. clamitans* larvæ.
3. Reciprocal, heteroplastic transplantation of potential dermal plicæ integument between *R. palustris* and *R. clamitans* larvæ.
4. Homoplastic transplantation of potential dermal plicæ integument of *R. palustris* larvæ to newly metamorphosed *R. palustris* frogs.
5. Heteroplastic transplantation of potential dermal plicæ integument of *R. catesbeiana* larvæ to newly-metamorphosed *R. palustris* frogs.
6. Homoplastic transplantation of differentiated dermal plicæ from newly-metamorphosed *R. palustris* frogs to *R. palustris* larvæ.
7. Heteroplastic transplantation of differentiated dermal plicæ from newly-metamorphosed *R. palustris* frogs to *R. catesbeiana* larvæ.

Results

Heteroplastic Transplantation of Potential Dermal Plicæ Integument

Three species of anuran larvæ were selected for the heteroplastic transplantation work, namely: *R. catesbeiana, R. clamitans,* and *R. palustris.* They were collected in the vicinity of Lake Winnisquam, New Hampshire, during the summer of 1932 in large numbers, making selection of various larval stages quite feasible. The *R. palustris* larvæ varied in length from 67 to 80 mm., with hind-limbs 5 to 35 mm. long; the *R. clamitans* larvæ being from 64 to 83 mm. in length, with hind-limbs 7 to 30 mm. long; while the *R. catesbeiana* larvæ were large, second-year forms 112 to 116 mm. long, with hind-limbs 9 to 15 mm. in length. In no case were larvæ used, however, in which histological signs of dermal plicæ structures had developed.

The technique of transplantation employed was similar to that as previously described by the writer (1931). This may be briefly stated as follows: Following chloretonization of the larvæ, a rectangular piece of integument was removed from the potential dorso-lateral dermal plicæ region of one individual and transplanted, heteroplastically, to the same region of another. Upon recovery from the anæsthetic, the larvæ were isolated in individual aquaria and observations of the grafts made during the subsequent metamorphosis of the host animals. In the case of *R. catesbeiana* larvæ, it was necessary to feed desiccated thyroid in order to induce complete involution. The *R. clamitans* and

R. palustris larvæ, however, usually underwent normal transformation. Following metamorphosis of the host animals, the transplants were removed for histological identification and examination of dermal plicæ structures.

Twenty-eight cases of complete metamorphosis were obtained using *R. palustris* larvæ to which *R. catesbeiana* integument had previously been transplanted. The time required for the attainment of complete larval involution (the time the transplants remained on the hosts) varied between 27 and 53 days. The dermal plicæ of the host animals developed normally and were well formed by the time complete involution of the latter occurred. The transplanted *R. catesbeiana* grafts, however, underwent a definite sequence of changes. The transplants healed readily and appeared quite normal for several days following grafting. Following this period, a general darkening of the grafts became progressively more evident; the transplants being quite black by the time complete involution of the host was obtained. A marked reduction in surface area of the transplants (from 30 to 50 per cent) also took place. In many cases definite erosion of the epithelium was also noticed. In the majority of cases there were no external signs of dermal plicæ formation, although in a few there were evidences of a faint light-colored stripe typical of early plicæ formation.

The histological examination consisted of serial sectioning of transplants together with a small amount of the adjacent dermal plicæ integument of the host. The skin of the host invariably showed normal, well-developed dermal plicæ structures. As for the transplants, no thickening of integumentary layers was ever observed. There was, however, definite evidence in several cases of a slight development of the mucous glands (see Fig. 1), characteristic of early dermal plicæ formation. That considerable degeneration of the transplants had taken

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EXPLANATION OF PLATE I

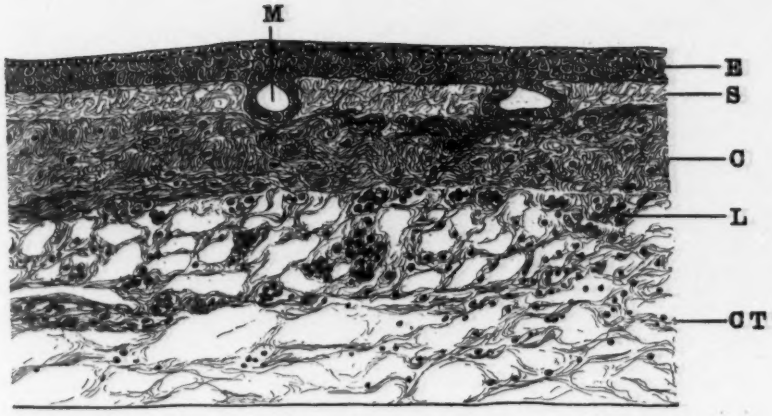
FIGS. 1 to 3. *BV*, blood vessel; *C*, stratum compactum; *CT*, subcutaneous connective tissue; *D*, duct of mucous gland; *E*, epidermis; *L*, lymphocytes; *M*, mucous gland; *P*, poison gland; *S*, stratum spongiosum; *X*, junction of transplant and host integuments, transplant to the left of *X*, host integument to the right.

FIG. 1. Histological section through *R. catesbeiana* integument on *R. palustris*, showing slight development of mucous glands and generalized histolysis.

FIG. 2. Histological section through *R. catesbeiana* integument on *R. clamitans* and adjacent integument of the latter. Note glandular development in host skin and absence of same coupled with pronounced histolysis in the transplant integument.

FIG. 3. Histological section through *R. palustris* dermal plicæ three weeks following transplantation to non-metamorphosing *R. palustris* larva. Note normal appearance of glandular structures and integumentary layers.

PLATE I



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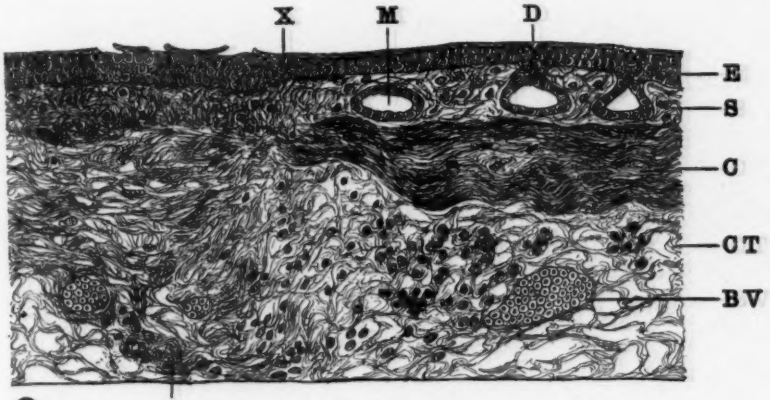

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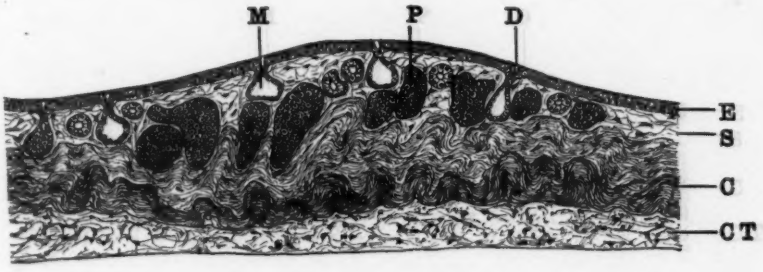

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place was evident by the large number of lymphocytes present in the subcutaneous connective tissue and the invasion of the stratum compactum by these cells.

The reciprocal transplantation of *R. palustris* integument to *R. catesbeiana* larvæ gave quite similar results. Twenty-four cases of complete host metamorphosis were obtained, the normal dermal plicæ of which were always well developed. The *R. palustris* transplants underwent progressive darkening and reduction in surface area, while no external signs of dermal plicæ formation were ever observed. Histological examination likewise failed to show even slight signs of glandular development or the formation of other dermal plicæ structures. Evidences of tissue histolysis and lymphocytic action were not as pronounced, however, as had been observed for the *R. catesbeiana* transplants.

Twenty-three cases of complete metamorphosis were obtained of *R. clamitans* larvæ to which *R. catesbeiana* potential dermal plicæ integument had previously been transplanted. The normal dermal plicæ of the host animals were undergoing development at the close of the involution period, although no external signs of such development were present in the transplants; which at this time had been on the involuting larvæ from 23 to 31 days. The latter also became quite black in coloration and reduced in surface area from 40 to 60 per cent. Histological examination gave evidence of early plicæ formation in the adjacent host skin, but none for the transplanted integument. Very pronounced signs of histolysis were present in the latter, resulting in complete disintegration, in many cases, of the stratum compactum layer and the associated infiltration of phagocytic lymphocytes (see Fig. 2).

Series of transplantations were also made of *R. clamitans* potential dermal plicæ integument to *R. catesbeiana* and *R. palustris* larvæ and of *R. palustris* integument to *R. clamitans* larvæ. Whether or not dermal plicæ development is possible following these heteroplastic integumentary transplantations was not determined, due to the fact that the host animals invariably died before metamorphosis had progressed far enough for the dermal plicæ of the latter to begin their development. The cause of death was not determined, although the inference might be drawn that the integumentary grafts exercised a toxic effect on the host animals. In all series, however, it was observed that considerable reduction in surface area of the transplants had taken place prior to the death of the host animals.

Transplantation of Potential Dermal Plicæ Integument to Newly-metamorphosed Animals

The formation of normal dermal plicæ structures proceeds steadily during the metamorphosis of the larva, resulting in distinct glandular and cellular developments by the time larval involution is completed. Following metamorphosis, however, there is a continued growth and development of the various dermal plicæ components accompanying the growth of the young frog. Whether these later developments are the result of influences still present in the metamorphosed animal, or whether the developments are simply the end results of earlier larval stimuli is an interesting point in question. Transplantation of potential dermal plicæ integument from the larva to the young frog should serve to answer these questions, since development of dermal plicæ structures under such conditions would indicate a continued influence on the part of the host, while failure of such development would serve to justify the reverse conclusion. The results of such transplantations are described below.

Thirty-six transplantations were made from *R. palustris* larvæ to newly-metamorphosed *R. palustris* frogs. The larvæ varied in length from 60 to 78 mm., with hind limbs 1 to 19 mm. long, while the young frogs were normal in all respects except for the persistence of small tail stumps 2 to 15 mm. in length. In making the transplantations, rectangular areas of integument from the dorso-lateral dermal plicæ regions were first removed from the young frogs and the potential dermal plicæ skin from the larvæ was then transplanted to these wound areas. The young frogs lived from 20 to 27 days following such transplantations, at which time the grafts were removed for fixation and subsequent histological examination.

In the preponderence of cases, a definite thinning of the transplants occurred, which was subsequently shown to be due to a progressive cellular histolysis of the various integumentary layers. In several cases, resorption of the grafts was so pronoûnced that large central areas were entirely eroded away. In such cases the histolytic processes were so intense as to affect the structure of the adjacent dermal plicæ of the host animal (see Fig. 5). Several cases of the latter condition were observed in which mucous and poison glands had undergone nearly complete degeneration, while the various integumentary layers, with the exception of the epidermis, were markedly affected. Three cases presented relatively little cellular degeneration of the transplanted integument. These gave external evidence of the formation of faint white-colored stripes, characteristic of early dermal plicæ formation. Upon sectioning, slight glandular development was evident, which, however,

never progressed to the extent of producing thickening of the stratum spongiosum.

Heteroplastic transplantations were also made from *R. catesbeiana* larvæ to young *R. palustris* frogs. The larvæ varied in length from 86 to 116 mm., with hind limbs 2 to 11 mm. in length, while the *R. palustris* frogs were similar to those used for the homoplastic transplantations. Of the twenty-eight transplantations made, twenty-six of the young frogs lived from 18 to 25 days following the operative period, while two lived for 37 and 39 days, respectively.

The *R. catesbeiana* integumentary grafts invariably assumed a darker coloration as compared with the adjacent host skin. There were also definite signs, in many, of epidermal erosion. In the majority of cases there were no external signs of dermal plicæ formation, although slight evidences of glandular development were usually seen upon sectioning. In several individuals (including the two cases living for 37 and 39 days following the transplantation period) definite ridges were formed. These ridges were quite characteristic of early dermal plicæ formation, although whitening of the ridge did not occur. Sectioning revealed pronounced development of mucous and poison glands with considerable hypertrophy of the stratum spongiosum (see Fig. 4). Even in these cases, however, considerable evidence of tissue histolysis was evident.

Transplantation of Well-developed Dermal Plicæ to Non-metamorphosing Larvæ

The stability of well-formed dermal plicæ structures, and their possible ability to continue growth when removed from influences of the metamorphosed animal, was determined by transplantation of dermal plicæ from newly-metamorphosed frogs to non-metamorphosing larvæ. Should such transplants maintain normal plicæ structures, the inference could be drawn that their stability is not dependent on influences prev-

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EXPLANATION OF PLATE II

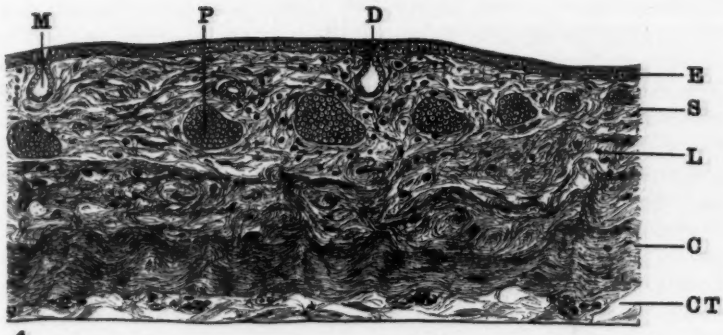
FIGS. 4 to 6. *BV*, blood vessel; *C*, stratum compactum; *CT*, subcutaneous connective tissue; *D*, duct of mucous gland; *DG*, degenerating poison gland; *E*, epidermis; *L*, lymphocytes; *M*, mucous gland; *P*, poison gland; *S*, stratum spongiosum; *X*, junction of transplant and host integuments, transplant to the left of *X*, host integument to the right.

FIG. 4. Histological section through *R. catesbeiana* integument on young *R. palustris* frog 37 days following transplantation. Note development of mucous and poison glands and dissociation of upper layers of stratum compactum.

FIG. 5. Histological section through *R. palustris* integument on young *R. palustris* frog 25 days following transplantation. Note pronounced histolysis of transplant and histolytic effect on adjacent integument of the host.

FIG. 6. Histological section through *R. palustris* dermal plicæ three weeks following transplantation to non-metamorphosing *R. catesbeiana* larvæ. Note absence of glandular structures and infiltration of large lymphocytes.

PLATE II



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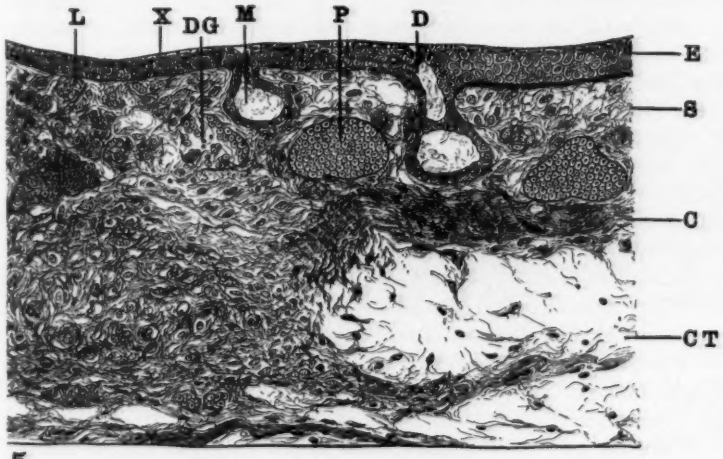

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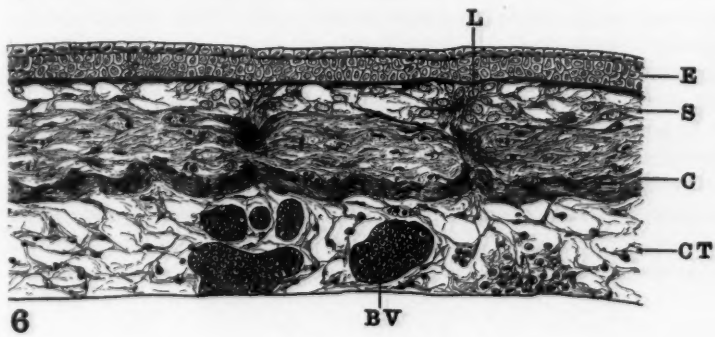

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alent in the young frog. Should regression of structures occur, the reverse conclusion could be proposed. The results of such transplantations are described below.

Sixteen transplantations were made of dorso-lateral dermal plicæ integument from newly-metamorphosed *R. palustris* frogs to R. *palustris* larvæ. The young frogs were completely metamorphosed with the exception of small tail stumps 5 to 11 mm. in length. The dermal plicæ of such individuals was well developed and appeared externally as a white, definitely ridged structure. Histologically, the plicæ were characterized by well-formed mucous and exceptionally large poison glands. The host larvæ were selected on the basis of small hind-limb growth, representing a stage of development in which metamorphosis would not begin for at least five or six weeks. The hind limbs of such individuals varied from 2 to 6 mm. in length, while the total length varied between 61 and 69 mm.

In general, the transplants underwent a gradual change in coloration from their original greenish hue to the almost black shade of the host integument. There was no evidence of a reduction in surface area, while the dermal plicæ underwent no external diminution either as regards size or visibility. Twenty-one days following transplantation, the fourteen larvæ that had survived were killed and the transplants removed for sectioning and subsequent study. Histological examination gave evidence that no reduction of dermal plicæ glands or integumentary layers had occurred. The histological appearance was practically the same as when transplanted (see Fig. 3). It should be noted here, however, that development of dermal plicæ structures never progressed beyond the stage already attained when previously removed for transplantation. Normally, on the newly-metamorphosed frog, a definite enlargement of dermal plicæ structures takes place within a three-weeks interval following larval involution.

A second series of transplantations involved the grafting of similar dermal plicæ integument from newly-metamorphosed *R. palustris* frogs onto non-metamorphosing *R. catesbeiana* larvæ. The donors in this case were in the same stage of development as described for the homoplastic transplantations, while the *R. catesbeiana* larvæ were second-year forms from 91 to 116 mm. in total length with hind limb buds 2 to 11 mm. in length. Such larvæ were not destined to metamorphose until the following summer.

Twenty transplantations were made in all. Fourteen of the host animals survived for three weeks, at which time the transplants were removed for sectioning. During the three-weeks interval on the host larvæ, the grafts underwent practically no reduction in area, although

a generalized darkening occurred until the coloration matched that of the adjacent larval integument. A very definite external diminution in the appearance of the dermal plicæ stripe took place, however, resulting in several cases in the complete disappearance of all macroscopic signs of the latter. Where the dermal plicæ stripe was still visible, it was usually much darker in coloration as compared with its original appearance. Histological examination of sections showed that a progressive histolysis of glandular structures, in particular, had taken place. Where the stripe was still visible, externally, the glands were found to be much reduced in size and undergoing degeneration. Where no external signs were apparent, usually all vestiges of glandular formations had disappeared (see Fig. 6). The lymphocytes were particularly abundant in such cases, while remnants of histolyzed structures could frequently be detected as far down as the subcutaneous connective tissue.

Discussion

The results of heteroplastic transplantations between larvæ indicate that while the grafts appear to heal and remain healthy for a time, cellular histolysis always occurs during the involution of the host animal. In this respect *R. catesbeiana* integument would appear to be less resistant than *R. palustris* integument, following reciprocal transplantation. Again, *R. catesbeiana* integument proved to be much less resistant to histolysis when transplanted to *R. clamitans* than when grafted to *R. palustris*. Obviously, the histolysis of heteroplastic integumentary transplants must be concerned with species specificity.

In general, it may be said that the failure of dermal plicæ to develop following such heteroplastic transplantations, was correlated with and probably due to inhibiting influences set up by the histolytic reactions. In this respect it should be noted that glandular development occurred only in cases where relatively little histolysis ensued, *i.e.*, in several transplantations of *R. catesbeiana* integument to *R. palustris* larvæ. It is interesting to note here, also, that due to the more rapid involution of *R. palustris* larvæ as compared with *R. catesbeiana* and *R. clamitans* larvæ, the dermal plicæ of the former species normally develop at a faster rate. This in turn would seem to indicate that the influences which stimulate dermal plicæ formation in *R. palustris* are more intense than is true of those present in the other two species. This difference, if true, may account for the development of glandular elements in *R. catesbeiana* integument transplanted to *R. palustris*. The results in general, however, indicate that dermal plicæ development is possible

following heteroplastic transplantation, providing the integumentary transplants do not undergo pronounced cellular histolysis.

The results of integumentary transplants made to young *R. palustris* frogs show quite conclusively that dermal plicæ development is possible in such cases. The fact that such development was more pronounced in *R. catesbeiana* integument than in *R. palustris* integument transplanted to *R. palustris* frogs calls for interpretation since one would expect the reverse to be the case. If we assume, however, as seems quite likely, that *R. catesbeiana* dermal plicæ are normally subjected to a less intense developmental stimulus as compared with the normal dermal plicæ of *R. palustris*, then development of the former should be more easily elicited as compared with that of the latter when under the more intense stimulating influence of *R. palustris*. Whether or not the developmental differences, as recorded, can be explained in this manner, the results in general indicate that post-involution growth and differentiation of dermal plicæ structures is due to a continued developmental influence on the part of the growing frog.

Transplantation of well-formed dermal plicæ to non-metamorphosing larvæ gave evidence of the stability of glandular and integumentary layers during the period of the experiment (21 days). This was true, however, only for the homoplastic transplantations. The pronounced, and in some cases total, degeneration of dermal plicæ structures following heteroplastic transplantation was due, no doubt, to the general histolysis of the transplants which occurred. It must be admitted, however, that in some cases the degree of general histolysis did not justify the pronounced dermal plicæ degeneration observed. One striking fact regarding the homoplastic transplantations was that no further growth or development of dermal plicæ structures had taken place during the three weeks interval on the larva, while the normal dermal plicæ of the donors had undergone considerable enlargement during the same time interval. These results, therefore, again emphasize the conclusion that dermal plicæ of the metamorphosed animal require and continue to respond to developmental influences present in the growing frog.

The various developments and regressions of dermal plicæ structures as described in the present paper are provocative of interesting possibilities concerning the persistence of potentialities for dermal plicæ formation. Since total regression is possible following heteroplastic transplantation, it would be interesting to see whether such transplants could again develop dermal plicæ structures in a suitable environment. Since the writer (1928) has previously shown that any integument placed over the annular tympanic cartilage will develop into tympanic

membrane during larval involution, it would be of interest to determine whether integument having first undergone complete dermal plicæ regression could secondarily develop into tympanic membrane upon suitable transplantation. The above and related questions are at present under investigation by the writer.

Summary and Conclusions

Heteroplastic transplantation of potential dermal plicæ integument was made between larvæ of three species to ascertain possible development of dermal plicæ structure during host metamorphosis. Results were as follows:

R. catesbeiana to *R. palustris*—slight development.

R. catesbeiana to *R. clamitans*—no development; grafts underwent considerable resorption.

R. palustris to *R. catesbeiana*—no development; slight resorption of grafts.

Homoplastic and heteroplastic transplantations of potential dermal plicæ integument were made between larvæ and newly-metamorphosed adults to determine whether the stimulus for dermal plicæ development is retained following involution. Results were as follows:

R. palustris to *R. palustris*—pronounced resorption of grafts in most cases; slight development in several cases where little resorption occurred.

R. catesbeiana to *R. palustris*—in general, pronounced development, especially of mucous and poison glands.

Homoplastic and heteroplastic transplantations of well-developed dermal plicæ integument were made from newly-metamorphosed frogs to non-metamorphosing larvæ to determine the stability of dermal plicæ structure or its possible regression. Abbreviated results were as follows:

R. palustris to *R. palustris*—no regression; typical structure maintained.

R. palustris to *R. catesbeiana*—very definite regression; no histological characteristics remaining in several cases.

In general, it may be concluded that development is possible following heteroplastic transplantation providing pronounced integumentary histolysis does not occur. Post-involution growth and differentiation of dermal plicæ structures is due to continued developmental influences present in the growing frog. *R. palustris* dermal plicæ resist regression following homoplastic transplantation but are susceptible to regression following heteroplastic transplantation to non-involuting larvæ.

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OBSERVATIONS ON THE PENETRATION OF DAYLIGHT INTO MID-ATLANTIC AND COASTAL WATERS

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Although considerable information on the penetration of daylight into the sea has been obtained from measurements made by Shelford and Gail (1922), Shelford (1929), Poole and Atkins (1929), Atkins (1932), and Atkins and Poole (1933), it was desired to extend this type of investigation to other regions and to study in further detail the effect of varying conditions upon submarine irradiation.[2] It was hoped to make observations in the open ocean, where the photo-electric method had never before been employed, and to compare the intensity of irradiation found there with that existing in coastal regions. As far as possible it was desired to learn to what extent the degree of light penetration is modified by changes in latitude, in time of day, and in condition of the sea surface. This information is of particular importance in relation to the theories for the vertical distribution of plankton (cf. Russell, 1927; 1931). We therefore proposed to measure the intensity of submarine irradiation at the same time that plankton hauls were being made. The application of our results to the theory of the effect of light on the diurnal migration of plankton is considered in a separate paper (Clarke, 1933).

The observations presented in this paper were made from the "Atlantis," the research vessel of the Woods Hole Oceanographic Institution. The photometric apparatus was put aboard at Plymouth, England, in July, 1931. Measurements of the penetration of light into the sea were made at thirteen stations on the trip across the Atlantic during the succeeding six weeks. The work was continued in July and August, 1932, when several series of observations were carried out in the Gulf of Maine and in Woods Hole Harbor.

[1] Contribution No. 26.

[2] By irradiation is meant the radiant flux density of all wave lengths (cf. Sawyer, 1933), as distinct from illlumination which refers to visible wave lengths only. The term "radiation" has formerly been used loosely for irradiation.

Apparatus

The apparatus for the measurement of submarine irradiation assembled for the "Atlantis" has been described in detail by Gall and Atkins (1931). Each photometer contains a potassium (sensitized with hydrogen) photo-electric cell. Two of the cells are of the vacuum type; the third is gas-filled. The photo-electric current is measured with a potentiometer or neon lamp outfit. Essentially, the method consists in measuring alternately the intensity of daylight falling on deck and that penetrating to various depths beneath the surface of the sea. Values are thus obtained for the submarine irradiation as percentages of the light at the surface. Absolute values of the intensity of light are derived by calibration of the instruments against a photometric standard.

With the original arrangement of the apparatus considerable trouble was caused by the fouling of the lines intended to operate the shutter of the submarine photometer and by the horizontal straying of the instrument due to the drift of the ship and the great sideward resistance of the lines and cables. Some improvement was produced by replacing one line by a heavy weight which opened the shutter. However, fouling and straying were still troublesome in rough weather and whenever working at depths greater than 50 meters. Accordingly, the No. 1 photometer was provided with a new four-conductor insulated cable and with an internal shutter which can be opened and closed electrically.

The circuit for the internal shutter uses two of the conductors of the new cable. The other two conductors are used for the photo-electric circuit. The new cable was made for the Woods Hole Oceanographic Institution by the General Electric Company. It is 300 meters long, covered with a very tough rubber jacket, and is capable of withstanding a strain of 168 kilograms without injury. The original five lines running to the photometer from the ship have thus been reduced to two, viz., the steel supporting cable and the new four-conductor cable. Fouling never occurs even in rough weather, and since the diameter of the new cable is only 1.7 cm., the sideward resistance of the whole apparatus is greatly reduced. During the work following these improvements the supporting cable rarely reached an angle with the vertical greater than 20°.

The original external shutter has been left attached to the photometer case for protection of the glass window when not in use. Just before the photometer is swung over the side, this shutter is tied open. The internal device was made from a Packard photographic shutter, the aperture of which is 9 cm. in diameter. The rubber bulb, which ordinarily operated the shutter, has been removed and a short lever arm

attached to the post which, when moved to the left or to the right, opens or closes the leaves of the shutter. The lever arm is arranged as the armature of an electro-magnet (see Fig. 1). The free end of the lever arm, which is attached to the shutter, swings to the left or to the right according to whether the left-hand pole or the right-hand pole of the magnet is activated. Each pole is wound with 500 feet of double silk insulated No. 30 copper wire. One side of each coil is grounded to the case (and hence through the sea water and supporting steel cable to the hull of the ship).

The operation of the shutter is controlled by a double throw switch conveniently located in the deck laboratory near the potentiometer. The ship's current is used to activate the coils of the electro-magnet (see Fig. 1). Closing the circuit momentarily suffices to open the shutter, or to close it. The shutter remains in the position in which it is left when the current is cut off. Since the current is allowed to flow for only a short interval, the coils may be greatly overloaded,

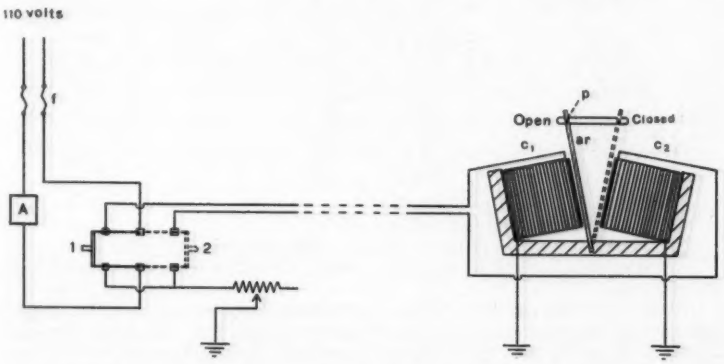

FIG. 1. Diagram of the connections of the electrically operated shutter.
(1) Position of switch to open shutter.
(2) Position of switch to close shutter. Except for the moments of operating the shutter, the switch is left with contacts broken in the vertical position.
A, ammeter; *ar*, armature; c_1, c_2, coils of electromagnet; *f*, 1-ampere fuses; *p*, post attached to leaves of shutter.

thus increasing the strength of the magnet. Ordinarily 0.4 ampere is sufficient to operate the shutter but the current may be increased to one ampere if necessary. This method has the additional advantage that no current is flowing through the electro-magnets while measurements in the photo-electric cell are being made.

Standardization of Photometers

Before the "Atlantis" sailed from Plymouth, the photometers were compared by Dr. W. R. G. Atkins with a vacuum potassium cell which had been standardized previously against an open carbon arc lamp (see Gall and Atkins, 1931, where the lowest and highest intensities of light which can be measured by our apparatus are stated also). The values obtained by him are summarized in Table I.

Birge and Juday (1932, p. 525) have pointed out that the meter-candle is not a suitable unit for the measurement of irradiation under

Table I

Standardization of photometers. Irradiation necessary to produce photo-electric current of 1 microampere at an anode potential of 60 to 63 volts.

Photometer	Carbon arc used as a standard *	1000-watt Mazda lamp used as a standard		Photo-electric emission produced by test lamp (used to check for changes in sensitivity at sea)
	Irradiation extending over visible range	Irradiation extending over visible range	Irradiation within range: 3460A°–5260A°	
	meter-candles	*meter-candles*	*microwatts/cm.²†*	
Deck	370	1610	135	4.4 μa
No. 1 Sea (1931)	870	2080	175	—
No. 1 Sea (1932)	—	3760	315	2.1
No. 2 Sea	232‡	750‡	63‡	—

* Values given by Gall and Atkins (1931).

† 1 Microwatt/cm.² = 10 ergs/cm.²/sec. = .0000143 gm. cal./cm.²/min.

‡ Values obtained immediately after glowing the cell for one second (see Gall and Atkins, 1931).

It will be observed that the ratios of the carbon arc and the Mazda lamp standardizations are not the same for the three photometers. Evidently the sensitivities of the photo-electric cells changed considerably during the year which elapsed between the tests.

The decreased sensitivity of No. 1 as used in 1932 was caused by the greater distance between the photo-electric cell and the photometer window. This change in position was necessary in order to make room for the new internal shutter.

water. This unit is misleading when applied to the visible part of the solar radiation penetrating beneath the surface because the energy spectrum has become so profoundly modified. Moreover, the radiation beyond the limits of the visible should be measured in addition, and this cannot properly be expressed in meter-candles. Whatever unit is used, the following precaution must be taken in order to obtain a correct standardization of the photometer: in evaluating the standard source only that region of its spectral emission may be considered which

occurs within the spectral range to which the photometer actually used is sensitive. Measurements made without this precaution give an erroneous idea of the submarine irradiation and the actual values obtained depend upon the type of source used for standardization. An illustration of this is to be found in Table I, where three standardizations of our photometers are compared. The values in meter-candles based upon the carbon arc differ widely from those based upon the incandescent lamp. The discrepancy is due to the fact that the whole visible radiation of the two standards has been measured and that these differ in spectral distribution, particularly in the region to which our photometers are most sensitive (see below).

Submarine irradiation may be properly expressed in cal./cm.^{2}/min., in ergs/cm.^{2}/sec., or in microwatts/cm.^{2}, provided that the spectral range measured is stated. In this paper calibration values are given in microwatts/cm.^{2} for the narrow range within which our photometers are sensitive. To facilitate comparisons between different investigations it is desirable to state the irradiation at any depth in the sea as a percentage of that just over the surface, the measurements being made in the same way. The values may then be ascertained readily in terms of any unit which was found convenient for the determination of the irradiation in air. This method is used in the present paper. The transmissive exponent,[3] k ("absorption coefficient"), can be readily derived from the percentage data by applying the usual formula:

$$\frac{I}{I_0} = e^{-kL}$$

where L = thickness of water layer (expressed in meters) in which the intensity is reduced from I_0 to I. Convenient tables for the calculation involved are provided by Sawyer (1931).

The spectral sensitivities of our photo-electric cells (without the cases, but with the diffusing discs in position) have been determined by the U. S. Bureau of Standards. The curves are reproduced in Fig. 2, where it will be seen that no significant differences exist. For purposes of standardization the range of sensitivity of the photometers was not considered to extend to the extreme limits of the curves, for to do so would result in the inclusion of a relatively large amount of the standard lamp's radiation in the yellow which would be responsible for a very small part of the photo-electric current. The limits of the range of sensitivity of the photometers were arbitrarily set at 3460A° and 5260A° respectively. This range extends 900A° above and below the point of

[3] It is the *vertical* transmissive exponent that is actually measured. The question of the direction of light rays beneath the surface has been discussed by Atkins (1932, p. 184).

average maximum sensitivity (4360A°) and includes over 96 per cent of the total area of each curve.

The standardization of each photometer was carried out against a 1000-watt Mazda "C" lamp, manufactured, seasoned, and rated by the General Electric Company. The data supplied give the irradiation

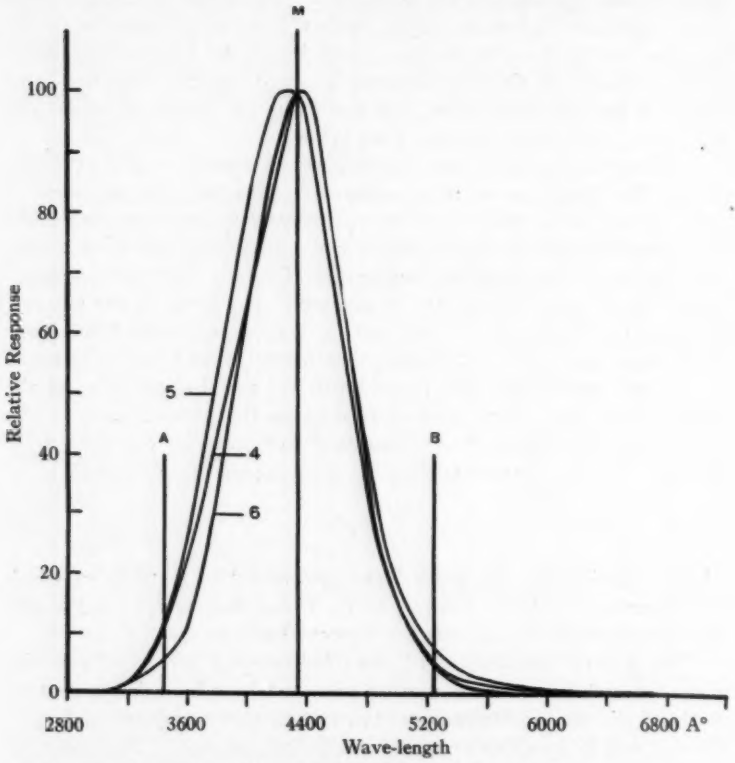

FIG. 2. Comparison of spectral sensitivities of photometers.

Curve 6: No. 7276—vacuum potassium (sensitized) cell—deck photometer.
Curve 5: No. 7275— " " " "—No. 1 sea photometer.
Curve 4: No. 7274—Gas-filled " " "—No. 2 " "

Each curve represents the relative response to an equal energy spectrum of the combined photo-electric cell and its diffusing disc of opal glass. For purposes of standardization the sensitivities are considered limited to the range between *A* and *B*, which are 900A° above and below *M*, the point of average maximum sensitivity.

$A = 3460\text{A}°$
$B = 5260\text{A}°$
$M = 4360\text{A}°$

present in a designated direction at a distance of one meter from the lamp (color temp. = 2835° K at 105.0 volts). The irradiation is expressed in microwatts/cm.^{2} for each wave length interval of 100A° from 3200A° to 10,000A°. From these data the irradiation at the photometer window (placed one meter from the lamp) within the designated range of spectral sensitivity of the photometers was calculated and found to be 126 microwatts/cm.^{2} By dividing this value by the photo-electric current produced, expressed in microamperes, the sensitivity of each photometer was found in terms of the irradiation necessary to produce a photo-electric current of one microampere. A summary of the standardization is given in Table I.

Changes in Emission of Photometers

Probably the greatest source of error during the measurements was the occurrence of changes in the emission of the photometers. It is unfortunate that there was no opportunity before the apparatus was assembled for testing the photo-electric cells in complete detail, as is recommended by Shelford (1930). When the "Atlantis" reached the United States and standardization was undertaken, the extent of the variability of the photometers was revealed. When the cells are irradiated in the darkroom by the standard lamp, the current emitted diminishes gradually during the course of the exposure.[4] This is particularly true of the gas-filled cell. This change of emission (formerly called "photo-electric fatigue") takes place chiefly within the first few minutes of exposure and rarely exceeds 10 per cent in magnitude (cf. Campbell and Ritchie, 1930; and Harrison, 1930).

Another more serious change in sensitivity was found to occur after an exposure of the photometer to intense irradiation (e.g. sunlight) for an hour or more. The response to the standard lamp following such treatment was greater—sometimes by as much as 30 per cent—than the response beforehand. Accordingly, when employed at sea, the deck photometer was probably less sensitive in the morning, after a night in darkness, than it was in the afternoon, after exposure to the noonday sun. Moreover, when either sea photometer had been used under water for an hour or more and then replaced on deck beside the deck photometer, it did not show the same ratio to the other instrument as before. It is hoped that in the future a satisfactory method can be devised for preventing the photo-electric current from surpassing the limit above which constancy is lost without reducing the range of intensities which can be measured.

[4] The potential was always applied continuously to the cells for at least ten minutes before readings were begun, thus avoiding any "soakage effects" in the cable.

During the observations of 1931, no method for checking the photometers existed except by comparing them with each other. However, there is no evidence that the error due to changes of emission ever exceeded 30 per cent and probably was rarely greater than 15 per cent. The deck photometer was almost always used with its diaphragm in place (see Gall and Atkins, 1931) so that the photo-electric current was never large. Most of the submarine measurements were made with the vacuum cell photometer No. 1, which has been found to be more stable than the others. The values obtained for submarine irradiation in terms of percentages of the light falling upon the surface are considerably more accurate than the absolute measurements. Since the deck photometer and the sea photometer were compared before and after each series, corrections for any changes in sensitivity could be made.

For the work of 1932 a portable test lamp, similar to that employed by Poole and Atkins (1928), was constructed. It consists of a seasoned 50-candle-power auto headlight bulb mounted in a copper shade which fits snugly over the photometer window. The photo-electric emission produced in each photometer by this test lamp (run at 5.00 volts) is carefully observed just after standardization (cf. Table I). Assuming that any changes observed to occur in the emission produced by the test lamp represent corresponding changes in the sensitivity of the photometer to daylight, the absolute value of the irradiation measured at sea can be calculated. The constancy of the test lamp is vouchsafed by checking against the standard lamp from time to time.

Other Sources of Error

As light travels through the water, its nature becomes changed in two ways: First, its intensity is reduced; second, its spectral range is diminished by the progressive elimination of the longest and the shortest rays.[5] Any photometer with a broad spectral sensitivity would accordingly register a reduction in illumination due to a combination of these two effects, and the parts played by each would be indistinguishable. This difficulty can be removed to some extent by placing suitable light filters over the cells, or by using cells which are sensitive to only a narrow range of the spectrum. As will be seen from Fig. 2, the present method falls in the latter category. It must be borne in mind, however, that nothing can be said as to the distribution of the irradiation within the range of sensitivity. The error from this source is fortunately small due to the relatively narrow and steep shape of the sensitivity curve.

[5] For the relative parts played by pure absorption and by scattering, see Martin (1922) and Hulburt (1932).

A more serious chance of misinterpretation results from the fact that observations on the degree of penetration of an individual color do not give an accurate measure of the change in total irradiation in the sea. Our photometers are sensitive to the group of wave lengths which is believed to penetrate the farthest in clear sea water (Grein, 1914; Hulburt, 1932; Atkins and Poole, 1933; and Sawyer, 1933).[6] Accordingly, the change in total irradiation with depth is greater than is indicated by the measurements in the present paper. Ideally, each color should be investigated separately, and then the results added together to provide quantitatively accurate values for total irradiation. Although limited regions of the spectrum are chiefly responsible for influence upon certain vital functions (e.g. photosynthesis; cf. Klugh, 1930), the whole spectrum—or a large part of it—is undoubtedly effective in many other cases.

The measurements presented in this paper are subject to a number of other possible sources of error, common to all observations of this type, the importance of which it is difficult to estimate. A brief enumeration may be made as follows:

(1) Errors due to the shape and diffusing quality of the receiving surface of the photometer, and to the occurrence of " dark currents " in the measuring circuit. This last trouble has been reduced to small proportions in our apparatus, due to the excellent quality of the insulation (Gall and Atkins, 1931).

(2) Errors due to changes in the cloudiness of the sky, in the elevation of the sun, and in the condition of the sea surface. Possibly the sea may be sufficiently smooth at times so that the light rays are not scattered at the surface to a significant extent, but under other conditions diffusion is undoubtedly so great that the surface of the sea is, in effect, the source of irradiation. Since the nature and the extent of the action of the surface under different conditions is not thoroughly known, and since in certain cases the angle of incident light has been found unimportant (Atkins, 1932, p. 184), no correction for the altitude of the sun has been attempted in this paper (cf. Birge and Juday, 1930). Whatever effects the surface is found to exert, the results will differ according to the method of measurement used, and, accordingly, great

[6] In determinations of the spectral absorption of sea water in which parallel rays chiefly are measured (cf. Sawyer, 1931) the rays of short wave length, which are scattered more than those of long wave length, are prevented by the apparatus from re-scattering back to the photometer as they do to some extent in measurements at sea. The transmission of the longer wave lengths would therefore appear relatively greater than under conditions where the scattered light was also measured. This fact should be borne in mind when making comparisons with other investigations. Suspended matter and stains may modify color selection profoundly (Shelford, 1929; Birge and Juday, 1931, 1932).

caution must be exercised in making comparisons with other investigations. In the present undertaking the values obtained for the upper water layers were checked in as many cases as possible with the neon lamp outfit (cf. Gall and Atkins, 1931).

(3)Errors due to the shadow of the ship and errors made in the estimation of the depth of the photometer. The ship was hove to with the stern directed as nearly as possible toward the sun, but in the work of 1931 it was not practicable to lower all the sails. The expanses of white canvas undoubtedly affected the deck cell, and in 1932 all the sails were lowered during measurements. The submarine photometer was suspended over the stern from the flagpole (in 1931) or from the end of the mizzen boom (in 1932). The heave of the ship caused a vertical movement of the photometer in the water, the amplitude of which was always noted (greatest extent, 3 m.), and the average depth recorded for each observation. This depth was corrected for horizontal displacement by multiplying by the cosine of the angle which the supporting cable made with the vertical for each observation. Since the cable probably was not straight under water but curved downward to some extent, this method of calculating the depth gives a slight over-correction (cf. Poole and Atkins, 1926).

(4) Errors due to the drifting of the ship into regions of water of different transparency in cases where horizontal distribution of suspended matter is irregular, and errors due to vertical changes in transparency caused by the diurnal migration of plankton. Observations on the actual effect of the plankton are discussed below.

These four possible sources of error are adequately discussed by Shelford and Kunz (1929), Shelford (1930), Poole and Atkins (1928, 1929), and Atkins and Poole (1933). There is no reason to suppose that the errors from these causes are any greater in this investigation than in those to which reference is made, and it is believed that the precautions taken, as outlined above, have actually reduced the magnitude of certain of the errors.

Observations

The observations made while crossing the Atlantic in 1931 are summarized in Table II. The complete curves for all of the series will be found in Figs. 3 and 4. The curves represent the irradiation at each depth expressed as a percentage of the light falling upon the surface and plotted on a logarithmic scale. The slope of each curve is therefore proportional to the value of its transmissive exponent. In each case the ratio of the photometers when the sea photometer was just over the surface is taken as 100 per cent. Measurements were ordi-

TABLE II

Summary of 1931 observations

Series No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Station No.	1002	1005	1007	1010	1013	1016	1019	1023	1029	1033	1038	1041	1053
Lat. N.	52° 17′	51° 17′	50° 09′	48° 27′	47° 04′	44° 59′	43° 13′	41° 45′	38° 13′	37° 27′	36° 56′	36° 55′	43° 27′
Long. W.	29° 46′	30° 03′	29° 57′	29° 58′	29° 59′	30° 08′	30° 10′	30° 33′	31° 39′	31° 44′	44° 52′	52° 41′	67° 39′
Date 1931	July 26	July 27	July 28	July 29	July 30	July 31	Aug. 1	Aug. 2	Aug. 4	Aug. 6	Aug. 12	Aug. 15	Aug. 24
Local sun time (24-hr. scale)	1133–1233	1235–1400	1205–1330	1130–1245	1115–1205	1110–1215	1100–1300	1103–1208	1253–1353	1223–1453	1545–1745	1055–1230	1010–1120
Av. altitude of sun	57°	46°	50°	60°	58°	60°	65°	61°	55°	54°	27°	64°	49°
Photometer No. *	1	1	1 N	1 N	1 N	1 N	1 N	1	2	1 N	1, 2	2	1 N
Av. transmissive exponent, k, over depth studied	0.10	0.10	0.061	0.058	0.051	0.052	0.049	0.055	0.044	0.053	0.046	0.031	0.12
Depth at which irrad. is reduced to 1%, *meters*	34	37	71	68	84	82	86	76	97	85	91	149	32
Range of irrad. on deck during series † (3460A°–5260A°), *microwatts/cm.²*	16,000–37,000	9,000–18,000	13,000–16,000	18,000–38,000	10,000–53,000	9,000–24,000	11,000–42,000	37,000–38,000	28,000–35,000	11,000–37,000	4,000–19,000	22,000–36,000	6,000–8,000
Sea (Douglas Sea Scale)	Moderate	Moderate	Moderate	Moderate	Moderate	Rough	Slight	Smooth	Moderate	Slight	Calm	Smooth	Rough
Sky	Clear	Overcast	Overcast	Few clouds	Many clouds	Many clouds	Few clouds	Clear	Few clouds	Few clouds	Few clouds	Clear	Overcast

* N in photometer column indicates that the measurements just under the surface and in the upper water layers were checked with the neon lamp outfit.

† Some of these values may be too high due to the probable increase in sensitivity of the photometer after use in very intense light.

narily made every ten meters. The value for each depth is the average of at least three readings. All measurements below the surface have been corrected for "external reflection" and "internal reflection" by multiplying by the factor 1.09 (cf. Atkins and Poole, 1933, p. 135).[7] The first point on the graph gives the percentage beneath the surface, at a depth (1 to 1.5 meters) just sufficient to prevent the photometer from coming out of the water when the ship pitched. The value obtained represents the reduction of light due to loss at the actual

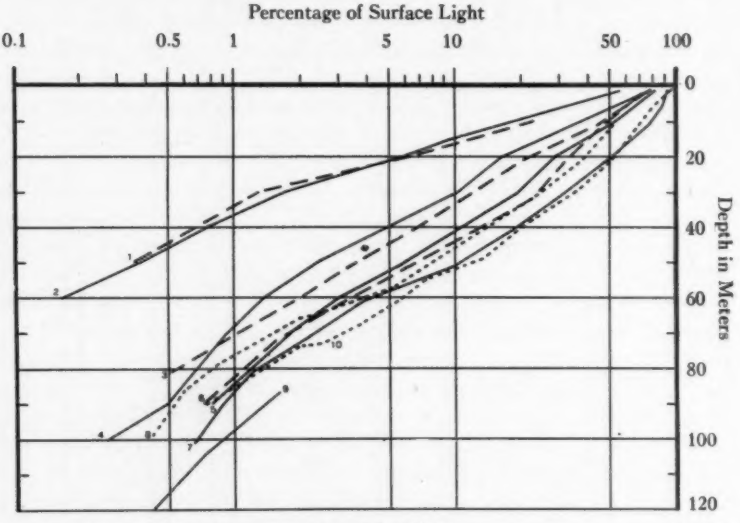

FIG. 3. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale). The series shown (1 to 10) were made in a line running south on longitude 30° W. The figure at the end of each curve designates the series number of that curve. The broken lines are merely to aid in distinguishing individual curves from each other in cases where they cross.

surface and to the absorption by the layer of water above the photometer.

The first ten series of observations were made on a line running north and south—approximately longitude 30° W. The observations were made in order from Series 1, the northernmost (lat. 52° N.), to Series 10, the southernmost (lat. 37° N.). It will be seen from Fig. 3 that the transparency of the water increases as one proceeds toward

[7] The use of this factor has resulted in values of 100 per cent or greater for certain of the measurements just beneath the surface. The source of error in these cases is not known.

the south. The average transmissive exponent is the same for the first two series ($k = 0.10$). For Series 3 the transmissive exponent is much reduced ($k = 0.061$). The value diminishes more gradually thereafter, reaching a minimum in Series 9 ($k = 0.044$). The values obtained in Series 5, 8, and 10 do not follow the sequence.

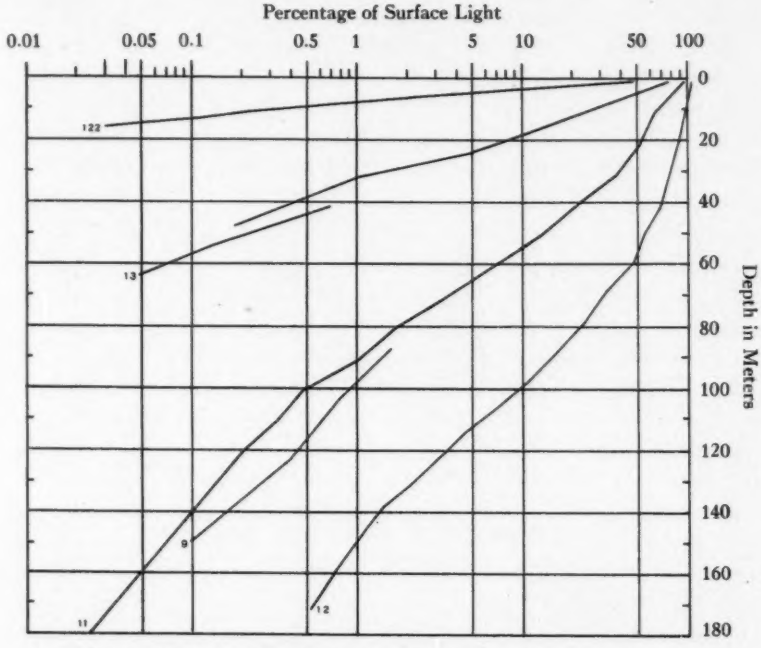

FIG. 4. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale).

Series 9—near Azore Islands.
Series 11 and 12—Sargosso Sea.
Series 13—Gulf of Maine.
Series 122—Woods Hole Harbor.

Series 12 and Series 122 represent respectively the most transparent water and the least transparent water encountered. The anomaly of the curve for Series 12 starting above 100 per cent is discussed in a footnote in the text.

The sudden reduction in the value of the transmissive exponent found between Series 2 and 3 is coincident with the sharp division between northern water and southern water as marked by the northern branch of the Gulf Stream. Toward the south the surface water is warmer and in addition the stratum of warm water extends to a much

greater depth (cf. Iselin, 1934). The lesser transparency of the northern water may be due to a greater amount of suspended matter (sediment and plankton) carried here from distant regions, or to a greater growth of plankton occurring in this region as a result of more favorable physical and chemical conditions.

A closer examination of the curves in Fig. 3 reveals that the differences in the transparency of the water at these ten stations are greatest within the upper 20 meters. In the first place great variation is found in the amount of light transmitted through the surface itself. The irradiation just under the surface (1 to 1.5 meters) varies from 57 per cent to 92 per cent. The lower values for the northern series may be due in part to the rougher weather experienced at those stations (cf. Atkins, 1932, p. 191). In the second place, the transmissive exponent of the subsurface water itself varies, for it is seen that the curves diverge rapidly from 1 meter to about 20 meters. From here on they are more nearly parallel. Since the sea was not smooth at any of these stations (except one), the change in altitude of the sun with latitude probably would not account for this change in transmissive exponent (cf. Atkins, 1932, p. 184). A greater amount of suspended matter in the upper 20 m. might account for the decreased transparency in this layer at the northern stations.

The curves for Series 11 and 12, made at stations 10° and 20° further to the west, are shown in Fig. 4. To facilitate comparisons with Fig. 3, the curve for Series 9 is repeated. The horizontal stray of the instrument was responsible for the fact that no greater vertical depth than 180 m. (calculated from wire angle) could be reached although the full 200 m. of cable was used. More irregularity was observed in the measurements at these great depths than normally characterized the shallower readings. This was undoubtedly due to fluctuations in the depth of the photometer which were not accurately reflected in the angle of the supporting cable. For the lower part of the series, the curves have been drawn as an average between the points rather than from point to point as in all other cases. The increased slope of the curve of Series 12 shows that the water at this station was more transparent ($k = 0.031$) than at any other station. However, the physical and chemical properties of the water were found to be the same as at the station where Series 11 was made (Iselin, 1934). The latitude of the two stations is practically identical. Since Series 11 was made in the late afternoon, whereas Series 12 was made at noon, the difference in elevation of the sun may have been responsible for the difference in transparency in this particular case. During both these observations the sea surface was smooth and probably did not diffuse the light nearly as much as in the cases discussed above.

The curves for the southern stations (Series 6 to 12) show a rather striking similarity in shape. The slope is steep at first, then becomes more gradual, and finally assumes a steep angle again. At the more easterly stations, the first change in slope occurs between 20 and 30 meters, the second change between 60 and 80. In Series 11 and 12 these changes fall respectively between 30 and 40 meters and between 100 and 140 meters. Evidently there exists an intermediate zone of decreased transparency which is found at greater depths at stations where the water is clearer. It is possible that this zone is similarly encountered at the northern stations, but that here it exists at a higher level and extends to the surface.

Series 13 was made in the Gulf of Maine under adverse weather conditions. The transparency is very slightly less than in Series 1 and 2. The remaining curve in Fig. 4 represents Series 122 made a year later in Woods Hole Harbor. The water here was found to be the least transparent encountered, light being reduced to 1 per cent at a depth of 8 meters. The curves of Fig. 4 therefore represent the extent of the range of transparencies observed during the two years work.

In 1932 eight series of observations were made in the Gulf of Maine. These are summarized in Table III. Curves plotted for the five series at Station 1287 are shown in Fig. 5. The loss at the surface, as indicated by the measurements at one meter, varies over a considerable range. Inspection of Table III shows that there is no simple relation between this loss and any one of the meteorological conditions. The value of this measurement probably results from a complex interaction of the elevation of the sun, the condition of the sky, and the state of the sea surface upon both reflection and refraction. However, the value of the transmissive exponent, from one meter down, varies very slightly during these four days. Diverse refraction at the surface may account for some of the differences observed, and the errors of measurement are probably responsible for others. The ship may have drifted into regions where varying amounts of suspended matter existed in the water. It is important to note that the diurnal vertical migration of zoöplankton has no measurable effect upon the transparency of the water in this case. The data presented elsewhere (Clarke, 1933) indicate the numbers of animals involved and the magnitude of their movements. The level of the zone of maximum abundance changes with the hour of the day. There are no corresponding changes in the transmissive exponent at the depths involved. Wherever steeper segments of the curve do occur, they do not correspond with zones of abundant zoöplankton.

Three series of measurements were made in Woods Hole Harbor from the power boat "Asterias." The data for these are presented in

TABLE III

Summary of Gulf of Maine and Woods Hole Series

Series	13	112	113	114	115	116	117	118	119	120	121	122
Station	1053	1285	1285	1286	1287	1287	1287	1287	1287	W. H. Harbor	W. H. Harbor	W. H. Harbor
Lat. N.	43° 27′	42° 34′	42° 34′	42° 30′	43° 04′	43° 04′	43° 04′	43° 04′	43° 04′	41° 31′	41° 31′	41° 31′
Long. W.	67° 39′	69° 37′	69° 37′	69° 37′	69° 24′	69° 24′	69° 24′	69° 24′	69° 24′	70° 40′	70° 40′	70° 40′
Date	Aug. 24 1931	July 9 1932	July 9	July 10	July 10	July 11	July 11	July 11	July 12	Aug. 16	Aug. 22	Aug. 22
Local sun time (24-hr. scale)	1010–1120	1302–1352	1907–1942	0652–0732	1526–1632	0805–0847	1203–1252	1837–1907	0612–0707	1149–1429	1029–1134	1339–1429
Av. altitude of sun	49°	64°	9°	16°	41°	29°	64°	15°	13°	61°	41°	50°
Photometer No.	1 N	1	1	1	1 N	1 N	1 N	1 N	1 N	1	1	1
Av. transmissive exponent, *k*, over depth studied	0.12	0.15	0.12	0.14	0.16	0.14	0.14	0.16	0.16	0.43	0.47	0.49
Irrad. at depth of 1 meter (% surface light)	78	100*	69	78	87	55		72	55	44	48	48
Range of irrad. on deck during series (3460A°–5260A°), *microwatts/cm.*^{2}	6,000–8,000	16,000–17,000	200–700	5,000–7,000	6,000–11,000	7,000–13,000	15,000–16,000	600–1,300	4,000–7,000	12,000–15,000	11,600–12,400	11,800–12,400
Sea (Douglas Sea Scale)	Rough	Slight	Moderate	Moderate	Slight	Rough	Moderate	Moderate	Slight	Calm	Calm (Glassy)	Calm
Sky	Overcast	Few clouds	Few clouds	Few clouds	Overcast	Overcast	Clear	Few clouds	Clear	Clear	Clear	Clear

* The explanation for this value, which is obviously too high, is not known (see footnote in text).

Table III and the curve for Series 122 is shown in Fig. 4. In this series measurements were made every meter to a depth of 16 meters. Since the water in the harbor is so rapidly changed by swift tidal currents, considerable fluctuations in the amount of sediment present undoubtedly occur. In Series 121 it was possible to measure the irradiation with the photometer suspended so that its window was only a

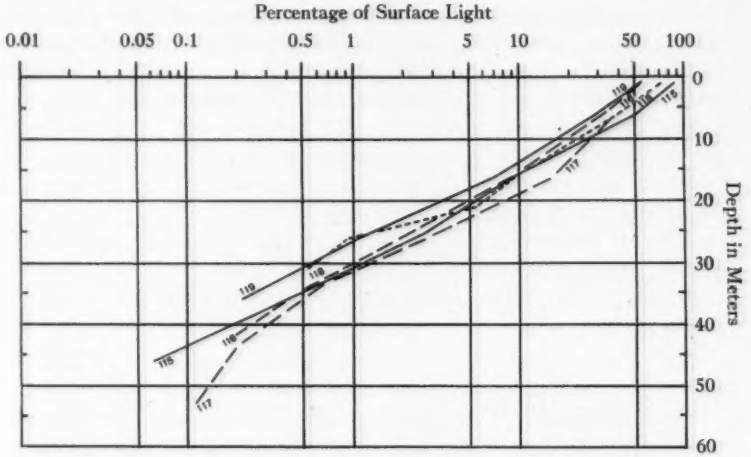

FIG. 5. Relation between depth and irradiation expressed in percentage of light just over the surface (logarithmic scale). The series shown (115 to 119) were all made at Station 1287 in the Gulf of Maine. The broken lines are merely to aid in distinguishing individual curves from each other in cases where they cross.

few centimeters below the surface. The irradiation was found to be 84 per cent of that recorded a few centimeters above the surface. Two hours later when Series 122 was made, a gentle breeze had sprung up and it was not possible to make another measurement as near the surface as before. The transmissive exponent for the whole series was slightly increased, but the irradiation at one meter remained the same.

DISCUSSION

A thorough review of the literature on the transmission of solar radiation through water has recently been made by Atkins (1932). Accordingly, no systematic comparison of the work of previous investigators will be attempted in the present paper. However, the general relationship between observations reported here and comparable investigations will be discussed briefly.

Comparisons can be made directly with the work of Poole and Atkins since our photometers have been standardized against theirs (see Table I). The values for surface loss vary within the same range in both investigations. The transmissive exponents found to characterize the water at Stations 1002 and 1005 and at Stations 1053 and 1287 are of the same magnitude as those observed by Poole and Atkins (1929) in the English Channel.

The measurements at stations farther to the south give transmissive exponents lower than any previously reported using the photoelectric cell. Employing photographic methods, Grein (1914) detected blue-violet light in the Mediterranean at 1500 meters; and Helland-Hansen (1931) found blue light at 500 meters in the Sargasso Sea.[8] Shelford and Gail (1922) reported blue light at 120 meters in Puget Sound amounting to about 0.2 per cent of the surface light. This indicates a transparency approximately equivalent to our Series 11. The transmissive exponent recorded for Series 12 ($k = 0.031$) approaches the value regarded by Atkins (1932, p. 185) as possible for the clearest ocean water. The values for the upper 59 meters in this series are:

meters	k
1–21	0.010
21–31	0.013
31–41	0.011
41–49	0.028
49–59	0.015

The transmissive exponent for the first 41 meters is lower than the minimum for pure water ($k = 0.015$) according to Sawyer (1931). This apparent anomaly may be due in part to the fact that Sawyer's measurement is diminished by the loss of light scattered in its passage through the water. In our measurements this loss is compensated to some extent by light which is scattered back into the cone of radiation received by the photometer. It does not seem likely that this effect could account for the whole discrepancy. Evidently, the measurements include some error. The fact that the value just under the surface is greater than 100 per cent also indicates this (see footnote above). However, one can hardly escape the conclusion that the transparency of the water at this station was exceptionally great—perhaps near the maximum possible.

The least transparent water observed—in Woods Hole Harbor—was found to have a transmissive exponent comparable to that reported

[8] See Atkins and Poole (1933, p. 154) for discussion of the conditions in the Sargasso Sea. Dr. Atkins informs me also that the color of the water on the Forel scale is 2 in this region as contrasted with 6 in the region where our first series was made.

by Poole and Atkins for certain measurements in the English Channel, especially those made near shore in the Plymouth region. The water of many of the Wisconsin lakes investigated by Birge and Juday shows a similar degree of turbidity. The transparency reported by Shelford (1929) for San Juan Channel is intermediate in value between that found in Woods Hole Harbor and that found in the Gulf of Maine. The present measurements, therefore, fall in line with those made by other investigators and, to a certain degree, extend our knowledge of the penetration of light into the sea.

Summary

1. Measurements of the penetration of daylight into the sea were made in the mid-Atlantic, in the Gulf of Maine, and in Woods Hole Harbor, using the photo-electric method described previously. A four-conductor insulated cable and an electrically operated internal shutter have been added to the No. 1 sea photometer.

2. All three photometers exhibit essentially the same spectral sensitivity. For purposes of standardization the range of sensitivity is considered to extend from 3460A° to 5260A° with a maximum at 4360A°.

3. The photometers have been standardized against a 1000-watt Mazda lamp, the value of the irradiation from which was limited to that occurring within the spectral range of the photometers. The results of standardization are presented in Table I.

4. The changes in emission of the photometers and other possible sources of error are discussed, particularly those not dealt with by previous investigators.

5. The observations made approximately on longitude 30° W. reveal an increasing transparency of the water, particularly of the upper 20 meters, toward the south. Toward the north, the transparency is slightly greater than that found in the Gulf of Maine (transmissive exponent, $k = 0.15$).

6. The most transparent water encountered was in the Sargasso Sea where measurements were made down to 180 meters. In Series 12 the average exponent was $k = 0.031$, and values of $k = 0.015$ to $k = 0.010$ were obtained in the upper water layers. The least transparent water was found in Woods Hole Harbor ($k = 0.49$).

7. Examination was made of the extent to which the altitude of the sun, the condition of the sea surface, and the vertical distribution of the zoöplankton affected the degree of penetration of light. Five series of observations under varying conditions at one station in the Gulf of Maine revealed no significant change in transparency.

8. The measurements of submarine irradiation are found to be commensurate with those of previous investigators, but certain significant differences are pointed out.

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THE POLYMORPHIC FORMS OF MELITTOBIA CHALYBII ASHMEAD AND THE DETERMINING FACTORS INVOLVED IN THEIR PRODUCTION. (HYMENOPTERA: CHALCIDOIDEA, EULOPHIDÆ)

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INTRODUCTION

The purpose of this paper is to describe the polymorphism which occurs in *Melittobia chalybii* Ashm.,[1] as observed in rearings made in the laboratory during the winter of 1928–29, and which, so far as known, has not heretofore been recorded for any species of *Melittobia*. This polymorphism involves the interpolation in the normal life-history of a generation of individuals which are genetically like the type-form but differ from that form in their structure and in their habits. In addition to the two forms of males and of females normally occurring, a third form of female was observed rarely. This form was also obtained under certain experimental conditions.

Melittobia chalybii resembles closely the European species *M. acasta* in its structural features, its habits, and its life-history. The latter insect has been quite thoroughly studied, in this country by Howard and Fiske (1911) and in Europe by a number of investigators, most recently by Graham-Smith (1919), Browne (1922), and Picard (1923). As a result of these studies the biology of *M. acasta* is now rather well known.

There is a decided structural antigeny, the males differing from the females in the reduction of their wings, the possession of an ocellus-like pigment spot on either side of the head in place of compound eyes, and in the modification of their antennæ.

The habits of *M. acasta* are also well known. It parasitizes the larvæ of numerous species of bees, wasps, and their hymenopterous parasites as well as the puparia of parasitic diptera in the nests of wasps and bees. In the laboratory it has likewise been reared upon the larvæ of ants and the pupa of a cerambycid beetle. This polyphagous habit of the species has been made in part the basis for a stimulating discussion on the problem of host relations by Thompson and Parker (1927).

Careful studies of the life-history of *M. acasta* have been carried

[1] The species was kindly determined by Mr. A. B. Gahan.

out, especially by Browne (1922) and Picard (1923), and the behavior of both sexes described in detail. The males are greatly outnumbered by the females, constituting only 5 per cent of the population. These males have excited the interest and the admiration of all observers by reason of their extraordinary courage and their ardor in love. They are very belligerent and engage in mortal combats with each other, not so much to gain the favors of the females but from sheer inherent pugnacity. Mating occurs within the cocoon or puparium, as the case may be, of the host and only the females eventually make their way to the outside. In the case of bees and wasps, female chalcids gain access to a host larva by entering the cells before they have been completed or they may, according to Thompson and Parker (1927), gnaw their way into the nest and into the cocoon of the host. Having found a host larva, the chalcid remains on its body, puncturing it by means of the ovipositor and in this way obtaining food and apparently also paralyzing it and preventing further development.

Hundreds of eggs are deposited upon the host in the course of the parasite's long life of three months, so that in spite of the small size of the chalcid larva (less than 2 mm.), a comparatively large host larva, such as that of one of the larger wasps, is completely devoured.

Parthenogenesis occurs, but virgin females lay only a few eggs which invariably develop into males. Such male offspring, according to Browne, mate with and fertilize the mother, which then begins to oviposit freely.

Buckell (1928) has described the life-history of *M. chalybii* from observations on several generations reared in the laboratory at Vernon, British Columbia, during the winter of 1926. The biology of this species was found to correspond in many of its details rather closely to that of *M. acasta*, as briefly outlined above.

In the present paper, therefore, lesser details of the life-history and of the behavior of *M. chalybii* will not be discussed at length, except in so far as they may be necessary for the purpose of this article, or as they differ markedly from those previously recorded for this insect.

Material and Methods

The chalcids used in this investigation were obtained from the mud-nests of *Trypoxylon politus* Say found on the walls in a deserted house at Goshen, Cape May County, New Jersey, in September 1928. Additional material was obtained from the nests of *Sceliphron cæmentarius* Drury and *Chalybion cyaneum* (Fab) collected near Doylestown, Pennsylvania, during December of the same year.

The host larvæ used in the experiments were found in the cells of

the above-mentioned nests and included *Trypoxylon*, *Chalybion*, chrysids, and an ichneumonoid parasite. In most of the work *Trypoxylon* served as the host although identical results were obtained with the use of the other species named.

All experimental rearings were carried out in small covered glass chambers or cells constructed for the purpose. It made no difference in the results when these were kept in the bright light on the laboratory table or in the dark incubator; nor is the maintenance of any degree of humidity a problem, since all larvæ transform perfectly whether they have been kept outdoors during the winter or have been allowed to lie entirely uncovered in the dry atmosphere of the heated laboratory.

The individual parasites used in starting the first series of experimental cultures were obtained from a single cocoon of *Trypoxylon* chosen from among many which were brought indoors during September. This cocoon, like many others, was fairly filled with the larvæ of *M. chalybii*, five hundred or more, all fully grown. Pupation, shortly followed by the emergence of adults, began towards the middle of November. Virgin females were readily secured by selecting and segregating pupæ of that sex, and all fertile females were obtained by mating such virgins, on the day they emerged, with individual males. In the first experimental series, there were employed ten of these females, all fertilized by a single male from the same host cocoon, and six other females which were left unmated. The mating of every fertile female used was observed under the binocular microscope. Each female was then placed in an individual culture chamber together with a host larva free from its cocoon.

Life History

An external examination of a *Trypoxylon* cocoon does not reveal whether or not it contains *Melittobia*. The material of the cocoon is hard, brittle and black, contains imbedded sand grains and is entirely intact. This indicates that the female chalcid gained entrance to the cell before the cocoon was spun and must have remained on the body of the host larva during the spinning process as suggested by Buckell and observed in *M. acasta* by Browne. On the other hand, Thompson and Parker state that *M. acasta* gnaws its way into the cocoon of its host and indicate that it also first bores its way through the mud wall of the nest.

Feeding

Soon after a female has been placed on a host larva feeding activities commence; punctures through the integument of the host are made

with the ovipositor, and subsequently these punctures are revisited and the minute bits of blood which have exuded are ingested. Previous writers have stated that some of these "stings" with the ovipositor are accompanied by the injection of a paralyzant which immobilizes the host and prevents its transformation. *Trypoxylon* larvæ during the winter diapause are extremely flaccid and are entirely incapable of any movement, paralyzation being therefore superfluous. As regards interruption of development of the host, it was found that the "stings" of the chalcid were not always efficient, at least not in the case of several larvæ from *Sceliphron* cocoons in which pupation, succeeded by the hardening of the ectoskeleton, intervened and prevented the feeding and development of the parasitic chalcids.

Feeding activities continue until the end of the female's life, usually a period of from 60 to 75 days. Whether her death ensues from old age or from her inability to obtain food from the host, which by that time has been rather completely consumed by her own offspring, was not determined.

Oviposition

By the eleventh or twelfth day the abdomen of the female has become greatly distended and oviposition begins. During this process the ovipositor does not penetrate the host, the point of it is merely pressed against the surface, and the egg, flowing out between the valves of the ovipositor, comes to lie on the surface of the host. In *M. chalybii* it was found that for the first three or four days eggs are laid at the rate of four or five per day. Whether this rate increases or decreases later on is not known, but it is safe to assert that a productivity of thirty-one eggs per day, as recorded by Browne for *M. acasta*, is not attained. *M. acasta* also differs in that it may begin oviposition within twenty-four hours after emerging.

Development

After an embryonic period lasting four days the young larvæ hatch and begin to feed on the surface of the host. The first few larvæ (from 12 to 20, when reared on *Trypoxylon*) then go through a very rapid development and emerge as imagoes within a minimum period of 14 days after oviposition. Some of them, it appears, may require a few days, perhaps as much as a week, longer. These imagoes, both males and females, are quite different from the type form of the insect in their appearance and in their behavior. The length of their imaginal life is also abbreviated, most of the females not surviving longer than three days, although some may live 15 to 20, or even 30 days. Never do

these individuals attempt to leave the cocoon of the host within which they were born, but after mating deposit their eggs upon the same host upon which their mother continues to oviposit. After the above-mentioned first few larvæ have transformed, the remaining larvæ derived from the eggs laid somewhat later by the original female, together with the larvæ hatching from the eggs laid by the short-lived females of the second-form (usually over five hundred or six hundred in number), experience a much longer period of development and do not emerge until about 90 days after oviposition. In Table I these two forms of individuals

TABLE I

Comparison of time of development and length of imaginal life of type-form and second-form of M. chalybii

Stadium	Type-form	Second-form
	days	*days*
Embryo	4	4
Larva	71	7
Pupa	15	3
Total	90	14
Longevity of the ♀	60–75	2–30

are compared. The figures for the type-form represent averages from a number of rearings; also they were not obtained from the observation of individual specimens but from the records of the stages present each day in the cultures. The figures for the second-form represent the average minimum periods; the total maximum would probably exceed the minimum by not more than a week.[2]

COMPARISON OF THE TWO FORMS

Imago

The outstanding structural differences between the two forms may

[2] Buckell (1928), in rearing several generations of *M. chalybii* at Vernon, British Columbia, indoors, during the winter of 1926 found that the times of development for the various stages were as follows: in the male, 3, 11, and 7 days for the egg, larva, and pupa respectively; in the female 4, 15, and 7 days. Browne (1922), from his rearings of *M. acasta* gives 2–9 days for the egg, and 8–9 days for the larva "under the most favorable conditions of food and temperature" and 7 days as the shortest pupal period, adding that both larval and pupal periods vary considerably in their duration.

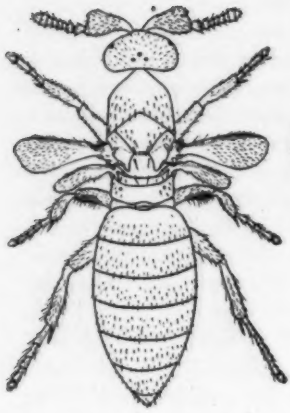
be understood by comparing the figures on Plate I. In the males of the type-form three well-developed dorsal ocelli are present on the vertex; while in the second-form these may be entirely absent in some individuals, or in others from one to three may be present in a more or less aborted condition and without pigment. The large, facetted eyes present in all females are aborted in both forms of the male, and are represented, in the type-form male, by a black ocellus-like spot on either side of the head. In the second-form male they are similar but the pigment is again absent and only the small oval windows in the integument can be discerned. The wings in the second-form, although completely expanded, are even smaller than in the type-form. In color the second-form males are of a dark reddish-brown, compared with the lighter brown of the type, a distinction which is most pronounced on the head.

In comparing the dimorphic females, it will be noted that in the second-form the wings remain practically in the condition in which we find them in the pupa, being short and crumpled. The insect never moves them. The abdomen of the second-form is very much enlarged and distended at the time of emergence. A thinner ectoskeleton in which the individual sclerites are often less completely differentiated, especially on the abdomen and in the antennæ where we may encounter a partial fusion of the joints, also characterizes the second-form. These females are brown in color while those of the type-form are a pitchy brownish-black.

The structural differences between the individuals of the type-form and of the second-form are associated with equally striking differences in their physiological characteristics and in their behavior. As regards the males of the type-form, it was found that they behave and carry out their life functions in a manner closely resembling the descriptions of previous authors for this species and for *M. acasta,* but with some exceptions.

The males of this species, as of the other, apparently do not feed at all and are unfriendly to other individuals of their own sex.[3] The act of copulation has been described in detail by Parker and Thompson.

[3] The terrible duels between males, described by a number of observers for *M. acasta* and by Buckell for *M. chalybii,* did not occur often. Even when a number of males were confined together in close quarters, with or without females, their encounters were generally without serious consequences. When two males happen upon each other they both kick their legs vigorously, as an insect does when becoming entangled in a web, and as a result tumble about awkardly, often rolling over together, but then quickly separate again as each goes on his way. Only in one instance did I observe two males wrestling together for a longer time. This encounter lasted ten minutes and ended only when one individual had succeeded in chewing off the left middle-leg of his antagonist, producing a large hole in the thorax at the point of attachment. This feature of the behavior of the males is mentioned here because it differs considerably in degree from that described for the same species by Buckell.



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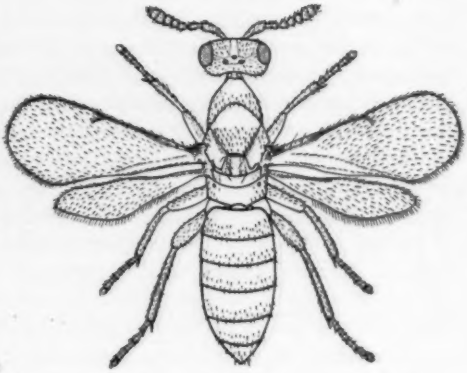

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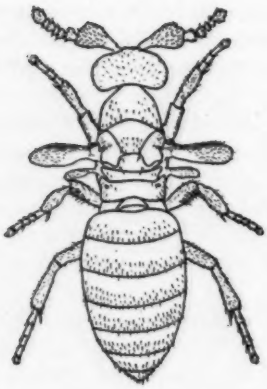

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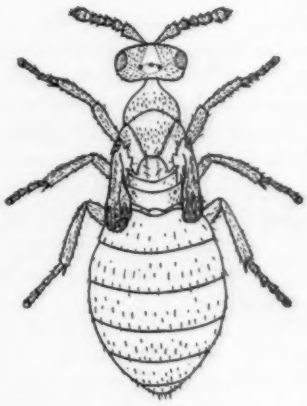


4

PLATE I

Melittobia chalybii Ashm.

FIG. 1. Type-form, male.
FIG. 2. Type-form, female.
FIG. 3. Second-form, male.
FIG. 4. Second-form, female.

In my observations, it was found to differ somewhat in the two forms. Preliminary movements go on for a period of two minutes followed by actual contact of the organs for about two seconds. The males of the second-form differ mainly in that the movements of their antennæ and legs during copulation are less regular and methodical, less perfectly coördinated, and that the entire act requires not more than one-half minute for its completion. This difference in the time given to the preliminary movements preceding insemination proved troublesome in experiments in which the males of one form were mated with females of the other form, since the coöperating final reactions of the female of each form are synchronized to the actions of the males of that form.

The males of both forms are comparatively short-lived; their constant activity and abstinence from food soon depletes their energy reserves and they die within a week after emergence.

Feeding and egg-laying activities as carried out by females of the type-form have already been described. It is these females which, after mating, make their way out of the cocoon and out of the nest of the host.[4]

The second-form of females is very different from the type-form in that the abdomen is very much enlarged at the time of pupation, the ovaries and eggs apparently developing during the pupal instar; and from 40 to 60 eggs may be laid on the day the adult emerges. The imperfections in the physical development of these females are paralleled by their very awkward and helpless behavior. They are barely able to crawl about slowly upon the host on which they were born; they mate and deposit their eggs in quick succession in the course of the first few days of their adult life. By the third or fourth day their abdomens become shrunken and collapsed as the supply of eggs is depleted and they die. Occasional females of this form are able to feed, and the peristaltic movements of their stomach are visible through the side of the abdomen. Such individuals may live and continue to oviposit for a period up to 30 days, the average being 14 days. In general, individual females are more apt to feed and continue to live if placed upon a fresh host where they are not disturbed by the presence of other adults and larvæ and where they can obtain nourishment more readily.

Larva and Pupa

Striking differences between the type-form and the second-form of

[4] Contrasting the type-form female of *M. chalybii* with the female of *M. acasta*, it is noted that in the former oviposition does not occur until the eleventh or twelfth day after feeding has begun. At the time of emergence the abdomen is rather slender, indicating that the ovaries have not yet fully developed, but after eleven days of feeding, the abdomen has become greatly distended and the insect begins to deposit eggs.

individuals are equally evident in the larval and pupal stages. The shortened life-history of the second-form involves, as we have noted, an acceleration of development in the larval and pupal stages, but not in the embryo. The larva grows with great rapidity and to a larger size than does that of the type-form. In seven days it may be fully developed, after which defecation occurs followed by pupation, the pupa of the second-form being easily recognized by the large, distended abdomen. Four days after pupation, the imago appears.

The larvæ which give rise to the type-form of the species remain as larvæ for a period of about seventy-five days [5] at room temperatures [6] during the winter.

Of this long larval period only the first week or two are given over to feeding, the actual time depending on the number of larvæ present and the condition of the host. During the remaining time the larvæ are in a kind of diapause, and it is this type of larva which hibernates in the cocoons of the host. A remarkable and puzzling circumstance is that although the host is always completely consumed by the parasite larvæ so that there remains only the shrivelled integument containing a quantity of white material, the urates of the fat body, these parasite larvæ are all perfectly developed. None of them appears undernourished and each without exception eventually transforms. Although the possibility of cannibalism among the larvæ is not entirely excluded, evidences of such behavior were not apparent. Neither form of larva spins a cocoon.

In both forms of larvæ, as in all other parasitic hymenopterous larvæ, the discharge of wastes and food residues occurs only once, just preceding pupation. In these chalcids, the egesta of the type-form consist, as previously described by Buckell, of a long dry chain of frass pellets, several times the length of the larva and gray in color. In the second-form the egested material is yellowish and semi-liquid, solidifying only after exposure to the air.

Discussion

When the life cycle as it probably occurs in nature is reviewed, its adaptive nature becomes immediately apparent. A female *Melittobia* of the type-form may enter the cell of a *Trypoxylon* before that cell has been sealed shut and remain there while the host larva is developing. During this time the chalcid may feed upon the larva or even upon the

[5] This time is the average of 20 cultures in which the date when the maximum number of eggs were present in each culture was noted and compared to the date on which the largest number of larvæ were pupating.

[6] The temperatures in the laboratory during the winter varied somewhat from day to day, ranging from 19° to 25° C., the usual temperature being 23° C., the room remaining at this temperature both day and night.

spiders with which the nest has been provisioned, and when the wasp larva spins its cocoon the chalcid becomes permanently enclosed within it and may then begin depositing its eggs. The parasite is very minute compared with its host and lays but four or five eggs per day, too low a rate to furnish sufficient offspring to consume the host entirely, especially if oviposition is begun late in the summer.[7] However, fourteen days after the first eggs have been laid, a generation of offspring consisting on the average of 13 females and 2 males of the short-lived or second-form of individual emerges. These remain within the cocoon and the females after mating each deposit from 40 to 60 or more eggs upon the same host. It is these eggs, together with the eggs the original mother continues to lay on this host, which give rise to the 500 to 800 larvæ finally produced, a number sufficient to use up entirely the available food. All of these larvæ, some the offspring of the original female but the majority the offspring of her daughters of the second-form, follow the longer course of development and after a diapause finally transform into imagoes of the type-form. It is larvæ of this kind which one finds hibernating in the cocoons of the host.

This interpolation of an additional generation of adults in the life-history is thus seen to constitute a remarkable biological adaptation which effects a more complete utilization of each host and, as a corollary, secures the production of a maximum number of offspring from each host. The structural characteristics of the two forms and their behavior also reflect the difference in the rôles they fulfill in the survival of the species. The type-form is produced in large numbers and is adapted to making its way out of the host cocoon, leading an active, "outdoor" life and seeking new hosts upon which to oviposit. While this form assures the dispersal of the species, the second-form is adapted only to life within the host cocoon chosen by its mother and its function is purely a reproductive one. Although the means used to secure the end are different, the final outcome of the production of this second-form of individual may be compared to the results of polyembryony in the life history of certain other chalcid flies.

Factors Conditioning the Production of the Polymorphic Forms

Life Cycle

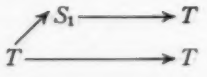
Under natural conditions, it is only the first few eggs laid by a type-form female which develop into individuals of the second-form; the

[7] From the length of the life history it appears that there are probably two generations of the type-form each year, one which emerges in the spring from the overwintering larvæ, and a second which emerges in the late summer.

other eggs as well as all of the eggs of the second-form females always develop into the type-form.

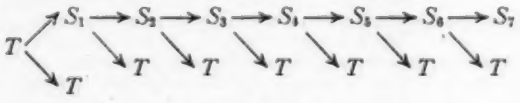
The questions which naturally arise are: Is there a difference in the eggs themselves which causes the first few to develop along a different direction from that followed by the eggs laid somewhat later, or are there other factors operating? Again, can individuals of the second-form produce only the type-form of insect, is there any regular alternation of forms involved, and are the two forms in any way genetically different?

In the ordinary course of the life cycle, the type-form female lays some eggs which yield second-form adults and others which yield again the type-form. If we let "T" represent the type female and "S_1" the second-form, then the succession of forms occurring in nature may be represented as follows:



However, if a female of the second-form is removed from the host upon which it was born and would ordinarily remain to deposit its eggs, and is placed upon a fresh host which has not yet been parasitized, we get the same end results as if we were using a female of the type-form. In other words, the first few eggs of this second-form female produce a generation of individuals like herself; the subsequent eggs, type-form individuals. Diagrammatically, we may represent the succession thus:

If we again remove the second-form imagoes to a fresh host we obtain the same results as before and this procedure can be continued, apparently indefinitely, so that we may secure a succession of generations of the second-form without the insertion of a generation of the type-form. This was actually carried through in the experiments, to the seventh generation. In the diagram each individual is shown giving rise, as it normally does, to type-form individuals also, as in the preceding description.



Crossing the Dimorphic Forms

Crosses were made in order to determine whether any genetic factors were involved in the production of the two forms and whether the males from eggs of virgin females [8] were in any way different from those produced by fertilized females. The various matings made are indicated in Table II, using the same notation as before. Of the crosses listed only the first and the fifth occur in nature in this species. " (V) " means that the male in question was the offspring of a virgin female.

TABLE II

Matings Made in Crossing Dimorphic Forms of M. chalybii

♀	♂	♀	♂
1. T	T	9. S_2	S_2(V)
2. T	S_1	10. S_2	S_3
3. T	S_1(V)	11. S_3	S_2
4. S_1	T	12. S_4	T
5. S_1	S_1	13. S_4	S_3
6. S_2	S_1	14. S_5	S_3
7. S_2	S_1(V)	15. S_6	S_5
8. S_2	S_2		

None of these rearings gave results which differed at all from those already described. They rule out any possible genetic mechanism as involved in this dimorphism, and rearings number 2, 4, and 12, which are among those that do not ordinarily occur, demonstrate that a female of either form mated with either kind of male may produce offspring of both forms.

Trophic Conditions

The fact that always only the first few larva which feed upon a particular host develop into individuals of the second-form while all others which may come after are destined to be of the type-form, suggested that possibly the food which the first larvæ obtain is qualitatively

[8] Virgin females feed upon the host larva as do the fertilized females and their abdomens become equally distended. However, very few eggs are actually deposited (from 0 to 10 in number). Of these the majority never hatch, but shrivel and dry up in the course of a few days. Occasionally, an egg will hatch and eventually develop into a male. It seems as if the proportion of eggs which hatch and produce males compared with the number which fail to develop is about the same as the ratio of males to females normally occurring in this species. From this follows the possible theoretical explanation that there is a predetermination in the ovary, that approximately 10 per cent of the eggs may develop parthenogenetically into males, while the remaining eggs cannot develop at all unless fertilized, when they produce females.

different from that available to the later ones. It is found to be invariably the case that after from twelve to twenty second-form larvæ have been reared on any *Trypoxylon* larva, it is no longer possible to rear anything but type-form larvæ upon that host. This fact was established by allowing females which had not yet oviposited to lay eggs on hosts from which a generation of second-form adults had already emerged. In such cultures, even the first eggs which the female lays yield only type-form adults. When eggs laid by a type-form female, after she has already produced the full number of second-form adults, were transferred to a fresh host, they too developed into offspring of the second-form, although if they had been allowed to remain on the original host they would have yielded only type-form individuals. Experiments in transferring larger numbers of eggs to a host entirely out of contact with adult chalcids rule out the possibility that a substance injected into the host by the ovipositing female may in some way affect the feeding larvæ.[9]

Other observations confirm the conclusion that there is a difference in the nature of the food taken up by the larvæ and that the food ingested by the first larvæ may be described as being richer. These observations are: (1) The larvæ of the second-form (those that feed first) grow more rapidly and attain a larger size. (2) Their excrement, as already indicated, is small in quantity and of a different color and consistency from that of the type-form, which is quite large in quantity and probably composed mostly of urates derived from the host. (3) When a smaller larva, *e.g.*, that of a chrysid, is the host, a correspondingly smaller number of second-form larvæ develop.

The conclusions then are, that those larvæ which are the first to feed upon a given host ingest the blood, while those which begin feeding somewhat later ingest to a larger extent the remaining available tissues, and that this trophic difference determines whether they will become adults of the second-form or of the type-form.

The Time and Nature of the Determination

In an attempt to gain some insight into the nature of the determining process and the time when it occurs, a few preliminary experiments were conducted. These consisted of transferring larvæ which had attained from one-third to one-half their full length from one host to another.

A. Larvæ from a fresh host were transferred to rather completely spent hosts on which several hundred larvæ had already fed. These

[9] Another but very remote possibility, namely that the larvæ which feed first may themselves inject substances into the host which affect the larvæ which feed after them, was not investigated. It would seem, however, that the larvæ producing such a secretion would themselves again ingest it.

larvæ, had they been allowed to remain on the first host, would have developed within 9 days into adults of the second-form.

Of 4 larvæ which were transferred, 1 completed its development on the second host in 29 days, yielding a type-form female, the other 3 died. In another experiment, again using 4 larvæ, the same result was obtained, except that the surviving individual required 30 days to complete its development. The death of most of the larvæ is ascribed to the difficulty they had in securing food from the used-up hosts and to the fact that decay had begun to set in in these used hosts.

B. Larvæ from a partly spent host were transferred to fresh, unused hosts. These larvæ, if left on the original host, would have required about 90 days to complete development and would have emerged as individuals of the type-form.

In one experiment, 4 larvæ after being transferred, all completed development within 12 days and emerged as females of an intermediate form, with wings somewhat shorter than in the type-form. In another trial, the time required was 13 days, for 2 of the 4 larvæ used. In a third, using 3 larvæ, 2 emerged on the twentieth day. All imagoes obtained were of the intermediate-form. The records for the last two experiments are incomplete due to interruption of the work.

Although the size and age of the larvæ were not carefully controlled and although the experiments were few in number, the records obtained do give some information regarding the dependence of the length of larval life and of the form of the adult upon the nature of the food taken in during different parts of the feeding period.

In the first series it is apparent that the shortening of the developmental period associated with feeding upon a fresh host is not lost when the larvæ are transferred to a used host, although the poorer food obtained from this second host does retard development by about 20 days. Either the trophic conditions during the latter part of the feeding period or the lengthening of the time of development, for the two are inseparable here, resulted in the production of individuals of the type-form.

In the second series, transferring larvæ to a fresh host accelerated their development so that they emerged, in most cases, as soon as if they had begun their feeding upon that host. But in spite of this rapid development and larger final size, characteristic for second-form individuals, the imagoes actually obtained resembled more closely those of the type-form. They are here designated as of an intermediate-form. The wings, although only two-thirds normal length, are fully expanded, as in the type-form; the abdomen is somewhat distended at the time of emergence but is not as large as that of the second-form, while in color they are again almost as dark as the type.

From these experiments it has become apparent that the length of larval life is directly determined by the trophic conditions obtaining, especially those obtaining during the latter half of the feeding period.

It appears probable (from Series B) that the production of the type-form is determined during the earlier larval period, but that this tendency will not be entirely realized if the entire larval period is shortened by transferring the larva to a fresh host. In this event, it appears as if the wings did not have time to grow to their full normal length during the very brief pupal stage, intermediate-forms resulting.

The development of second-form adults seems to depend both upon the early trophic conditions and upon the shortening of the larval and pupal periods. More definite or detailed conclusions cannot be ventured until more extensive experiments have been made.

Conclusion

The polymorphic forms in *M. chalybii* have been found to differ from each other both morphologically and physiologically and have been shown to be conditioned by extrinsic trophic factors. Both in the existence of structural differences between the forms and in the nature of the factors which produce them, the polymorphism in this chalcid is therefore comparable to that occurring in the social bees and wasps.

In *Melittobia,* as in the social forms, the food is not to be considered as a direct cause of the differences between the polymorphic forms, but rather as a factor which acts in an indirect way upon the growing larva and determines which one of two inherently possible paths of development will be followed. The restriction of the insect to one of two alternatives in development is especially obvious in species like *Melittobia chalybii* and the honeybee, in which intermediates between the two forms of the female are exceedingly rare, in spite of the fact that, at least in the former species, all trophic gradations from the most to the least favorable are experienced by numerous individual larvæ. In bumblebees, on the contrary, we find less difference between the two forms of the female, and the intermediate forms seem to indicate a more direct dependence upon the amount or the quality of the food received by the larva.

Finally, it is of interest to recall here that in one of the few other instances of polymorphism in chalcids, that of *Trichogramma cacœciæ,* an egg parasite, Marchal has found that the larvæ are also sensitive to food differences and react by a change in the rate of development, this rate depending upon the rate of development of the host embryo.

SUMMARY

1. The parasitic chalcid *Melittobia chalybii* Ashm. occurs in two forms, the type-form and the short-lived or second-form. The production of one or the other of these two forms is determined by the trophic conditions obtaining during the growth of the larva. A third form of female was obtainable experimentally and very rarely in the normal cultures.

2. The males of the second-form differ from the type-form in their darker color and in the more aborted condition of their optic organs. The females of the second-form differ from typical females in their lighter color, in the failure of their wings to spread upon emerging from the pupa, in the large swollen condition of the abdomen which is full of ripe eggs at the time of emergence, and in the shortness of their imaginal life. Both sexes of the second-form emerge as adults in a minimum of fourteen days after oviposition compared with the ninety days required for the development of individuals of the type-form.

3. The life history is as follows: A female of the type-form enters the nest of a host wasp (*e.g., Trypoxylon politus* Say), and remains upon the host larva within the cocoon, feeding and ovipositing for about 70 days, until it dies. The first few (12 to 20) eggs laid develop in about 14 days into a generation of males and females of the second-form. These do not leave the host cocoon and the females deposit their eggs upon the same host upon which their mother continues to oviposit. All eggs, those of the mother and those of her offspring of the second-form, now develop in about 90 days into chalcids of the type-form. After mating, the females alone gnaw their way to the exterior.

4. Experiments involving the transfer of eggs from one host to another and the transfer of ovipositing females to different individual host larvæ demonstrated that all eggs of both forms of females are potentially alike and capable of developing into imagoes of either form. In every experiment, the first few larvæ which feed upon a given host invariably give rise to adults of the second-form, all the remaining larvæ to adults of the type-form. The conclusion is that the first larvæ ingest mainly the blood of the host while the later ones must feed to a larger extent upon the remaining tissues; and that this trophic difference determines the production of one or the other form of adult from a single type of egg.

5. Experiments involving the changing of the trophic conditions during the life of an individual larva give some insight into the nature of the determination and the time at which it occurs.

6. When larvæ were transferred from a partly consumed host to a

fresh host, a third form of female individual, termed intermediate-form, was obtained.

7. In crossing females of one form to males of the other form, including males derived from virgin females, the results obtained differed in no way from those obtained from the usual matings which occur in nature.

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PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS

PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

June 30, 1933

EFFECTS OF CENTRIFUGAL FORCE ON THE ECTOPLASMIC LAYER, NUCLEI, AND PROTOPLASM OF FERTILIZED SEA URCHIN EGGS. Ethel B. Harvey.—The ectoplasmic layer of *Paracentrotus lividus, Parechinus microtuberculatus* and *Sphærechinus granularis* can be thrown down by centrifugal force as a ring which lies in the perivitelline space. When eggs kept in Ca-free sea water are centrifuged, the ring is not thrown down, but when returned to sea water, the ectoplasmic material is precipitated as small refringent granules in the perivitelline space.

If centrifuged before or during the monaster stage, the pronuclei can be separated, the female going to the light pole and the male to the heavy pole. In greatly elongated eggs, during the time that the male nucleus is approaching the female, it may enlarge so as to become three times the volume of the female. In eggs separated into two parts, one may contain the female and the other the male nucleus. In most cases, the female nucleus forms a monaster, and the fragment does not divide; the male nucleus forms an amphiaster and this fragment divides normally. In a few cases, the part with the female nucleus divided normally and the part with the male nucleus did not divide, the male nucleus grew smaller and disappeared.

Fertilized eggs (30–90 seconds after insemination) stratify and break into parts similar to unfertilized eggs, but slightly more elongate. During the monaster stage they form long streamers sometimes 14 times the original diameter of the egg. They elongate less during the streak stage and only slightly during the amphiaster stage. The fertilized eggs of *Parechinus* and *Paracentrotus* break more rapidly at all stages than the unfertilized, the fertilized eggs of *Sphærechinus* less rapidly than the unfertilized.

MOTION PICTURES TAKEN THROUGH THE CENTRIFUGE-MICROSCOPE ILLUSTRATING DIFFERENCES IN BEHAVIOR OF UNFERTILIZED AND FERTILIZED SEA URCHIN EGGS. E. Newton Harvey.

SOME DATA FROM A CORRELATED ANATOMICAL, PHYSIOLOGICAL AND BEHAVIORISTIC STUDY OF THE REPRODUCTIVE CYCLE IN THE FEMALE GUINEA PIG. William C. Young. (Published in the *Am. Jour. Physiol.*, **105:** 393–398, 1933.)

HÆMOGLOBIN-RINGER, A NEW MAMMALIAN PERFUSION FLUID. William R. Amberson.

July 5, 1933

SOME EFFECTS OF BLUE-GREEN ALGÆ ON LAKE FISH. G. W. Prescott.

DISTRIBUTION AND ECOLOGY OF THE MARINE ALGÆ OF THE MARITIME PROVINCES OF CANADA. Hugh P. Bell.

DISTRIBUTION OF THE FRESHWATER ALGÆ OF NEWFOUNDLAND. W. R. Taylor.—On the basis of three sets of samples examined by the writer, and a short list published by another investigator, about 500 desmids and 175 other algæ are recorded. Examination of the data for the desmids showed that they followed, in general, the distribution known to be characteristic of the Phanerogams. The

special occurrences of non-desmid algæ are discussed. The extended paper is to carry in addition to this information, detailed measurements, critical taxonomic notes (including over 30 new species, varieties, and forms), and illustrations of approximately all the desmids.

July 10, 1933

THE CONCENTRATION OF ORGANIC DERIVATIVES IN SEA WATER, IN RELATION TO THE CHEMICAL COMPOSITION OF PLANKTON. A. C. Redfield.

DIURNAL MIGRATION OF PLANKTON IN THE GULF OF MAINE AND ITS CORRELATION WITH CHANGES IN SUBMARINE IRRADIATION. George L. Clarke.

THE RÔLE OF BACTERIA IN THE FORMATION OF NITRATE IN THE SEA. Selman A. Waksman and Cornelia L. Carey.

July 14, 1933

THE EFFECT OF RESPIRATORY POISONS AND METHYLENE BLUE ON CLEAVAGE OF CERTAIN EGGS. M. M. Brooks.—CO (about 97 per cent) hindered cleavage of fertilized *Arbacia* and *Asterias* eggs as compared with N_2; an 80–20 mixture of CO and O_2 had little effect. KCN (5×10^{-5} M) inhibited cleavage (*Arbacia*). Methylene blue 5×10^{-6} per cent accelerated and 5×10^{-4} per cent retarded cleavage in *Arbacia; Asterias* behaved likewise but at lower concentrations. Methylene blue, 5×10^{-5} per cent antagonized the effects of CO and dilute KCN on cleavage, but not that of N_2, *i.e.*, lack of O.

IONIC CHANGES DURING THE DEVELOPMENT OF FISH EGGS. Laurence Irving and Jeanne F. Manery.

THE TENSION AT THE SURFACE OF EGG CELLS. E. Newton Harvey. (To be published in the *Jour. Cell. and Comp. Physiol.*, October, 1933.)

FERTILIZATION MEMBRANES OF CENTRIFUGED ASTERIAS EGGS. Donald P. Costello.

THE KIDNEY TUBULES WITH PHENOL RED AND NEUTRAL RED. Robert Chambers.

July 18, 1933

CHROMATIN EXTRUSION IN CERTAIN CILIATE COMMENSALS OF MUSSELS. G. W. Kidder.

THE REORGANIZATION BANDS IN THE MACRONUCLEUS OF ASPIDISCA. F. M. Summers.—The reorganization bands (Yocum, 1918) in *Aspidisca lynceus* Müll. are somewhat similar to those found in *Euplotes* (Griffin, 1910; Yocum, 1918; Turner, 1930), so that the same terminology has been adopted as far as possible.

The first indication of approaching division is the appearance of the reorganization band, which soon divides into two parts. These two bands, each consisting of three distinct planes, make their appearance (?) in the central portion of the C-shaped macronucleus and each traverses its respective half of the nucleus from the midregion to the extremity, where it disappears leaving the nucleus in a greatly altered condition.

The growth of the central region and the simultaneous disappearance of the older segments are apparently correlated with the migration of the reorganization bands through the macronucleus. Structural modifications coincident with reorganization are easily observed since both altered and unaltered parts of the nucleus are present for a time in the same organism. In Feulgen preparations the chromatin reticulum of the altered part is heavier, more compact, and with fewer

alveoli than that of the unaltered parts. Flemming-hæmatoxylin preparations demonstrate certain "pocket" granules in the distal regions that are not to be found in the reconstructed part.

Following Turner's (1930) interpretation of the phenomenon in *Euplotes patella* it is suggested that the three planes of each band represent three successive phases in the transformation of the central chromatin reticulum into non-staining nuclear substances. The non-staining phase (the reconstruction plane) may be a region of interplay between altered and unaltered parts of the nucleus and between these and the cytoplasm.

COMPARATIVE STUDIES ON THE OSMIOPHILIC AND NEUTRAL-RED-STAINABLE INCLUSIONS OF THE GENUS VORTICELLA. Harold E. Finley.—Osmic acid and silver nitrate techniques were used to prepare whole mounts and sections of *Vorticella convallaria* (Linnæus 1758), *V. microstoma* Ehrenberg 1830, and *V. campanula* Ehrenberg 1831. Neutral red and Janus green B were used as vital stains. Observations indicate that very few significant differences exist between these species of *Vorticella*, so far as osmiophilic and argentophile and neutral-red-stainable inclusions are concerned. Some possible sources of errors in the identification of the "so-called Golgi material" in the Protozoa are pointed out.

EXPERIMENTAL CYTOLOGY OF AMŒBA PROTEUS. S. O. Mast and W. L. Doyle.—Amœba proteus contains among other structures: fat globules; small bodies, usually elongated; truncated bipyramidal crystals, frequently with blebs attached; and highly refractive globular bodies held by different investigators to be secondary nuclei, nutritive spheres, Golgi bodies, and vacuome.

We found the following: The small bodies have all the essential characteristics of mitochondria. The crystals are probably a magnesium salt of a mono- or di-substituted glycine. There are two kinds of refractive bodies, one in the food vacuoles, the other free in the cytoplasm. The former is optically homogeneous. The latter consists of a surface layer of viscous substance on a fragile shell of carbohydrate material which surrounds a central plastic mass.

In attempting to trace the transformation of food into protoplasm, the components of the cytoplasm were stratified by centrifuging and held in this condition by chilling, then the various structures mentioned above were partially or entirely cut out, after which the specimens were either starved or fed with selected food. The following was found:

The crystals and the homogeneous refractive bodies form in the food vacuoles. The food vacuoles divide, forming small vacuoles each containing a crystal. The mitochondria pass from the surface of the food vacuoles to the crystal vacuoles. As this occurs the homogeneous refractive bodies and the crystals become smaller and the blebs on the crystals larger until the crystals disappear and the blebs become free refractive bodies. The mitochondria appear to transfer substance from the food vacuole to the crystal vacuole. The free refractive bodies have staining characteristics of plastids in plants. They are reserve food. Both types of refractive bodies are stages in the transformation of food into protoplasm.

July 25, 1933

ONE STEP IN THE DEVELOPMENT OF HEREDITARY PIGMENTATION IN THE FISH ORYZIAS LATIPES. H. B. Goodrich and C. B. Crampton.

TRANSLOCATIONS IN THE MOUSE AND THEIR EFFECT ON DEVELOPMENT. George D. Snell.

A SERIES OF PROBABLE MUTATIONS IN DROSOPHILA PSEUDO-OBSCURA AS COMPARED WITH D. MELANOGASTER. D. E. Lancefield.

SEX-DETERMINATION IN HYMENOPTERA. P. W. Whiting.—It has been shown in mosaic males of *Habrobracon* that the dominant eye color may appear at the

margin separating the genetically different tissues, each of which bears a different recessive factor. Thus black is reconstituted along the line separating ivory from white. Similarly feminized genitalia may occur if the two types of tissue adjoin in the genital region. Thus each of the two types of tissue in the male mosaic are recessive for a different factor determining sex. On the basis of these and other findings already published, it is postulated that females are in chromosome constitution, 1 $X+1$ $Y+2$ A, while normal (haploid) males are 1 $X+1$ A or 1 $Y+1$ A, and biparental (diploid) males are 2 $X+2$ A or 2 $Y+2$ A. X contains the factors F and g while Y contains f and G. The theory brings the problem of sex determination in Hymenoptera into line with current views of genic balance.

August 1, 1933

THE EFFECTS OF FAT SOLVENTS UPON THE FIXATION OF MITOCHONDRIA. Conway Zirkle.

STUDIES ON THE CYTOLOGY OF AMPHIBIA. Arthur W. Pollister.—A study has been made of the cytoplasmic structures in a variety of amphibian tissues, nearly all types being represented with the exception of the striated muscle fiber and the neurone. The Kull, Kolatchev, and Benda methods were chiefly employed. Every type of cell was found typically to contain three types of cytoplasmic structures: chondriosomes, usually in the shape of unbranched filaments; Golgi material, in the form of thin lamellæ; and, in nearly every type, a pair of centrioles. In the cells that are not physiologically polarized the Golgi platework always envelops the central apparatus that surrounds the centrioles. In cases where the cell contains an abundance of cytoplasm an aster is developed about the central apparatus, and chondrioconts in that part of the cell are oriented radial to the centrioles. To this unpolarized type belong the following cells: leucocytes, erythrocytes, mesenchyme cells, fibroblasts, gonial cells, peritoneal epithelial cells, endothelial cells, and smooth muscle fibers. In all physiologically polarized cells, *i.e.* columnar, cuboidal, and polyhedral epithelia, by contrast, the Golgi material has no fixed topographical relationship to the centrioles. The latter are usually located near the center of the distal end of the cell. The axis passing through the two is often perpendicular to the cell surface, and in some types a flagellum is attached to the distal centriole. The Golgi apparatus in most cases is in the form of a thin irregular belt, often surrounding the distal end of the nucleus. In some instances it is in the form of separate discoid lamellæ in the same position as the other type. In glandular cells the Golgi material is localized at the site of synthesis of the secretory product. In all the polarized cells the chondrioconts are oriented with their long axes parallel to the direction of the flow of materials through the cell, *i.e.* between the base and the lumen. It is suggested that cytoplasmic currents are responsible both for this arrangement and for the radial orientation of chondriosomes in the unpolarized cells.

THE IRRADIATION OF BIOLOGICAL SUSPENSIONS BY MONOCHROMATIC LIGHT. (THE EFFECT OF ULTRA-VIOLET LIGHT ON A PLANT VIRUS AND BACTERIA.) B. M. Duggar and Alexander Hollaender.

MOTION PICTURES SHOWING SOME VARIETIES OF NERVE IRRITATION, AS SEEN IN LIVING FROG TADPOLES. Carl C. Speidel.—The reactions of single internodal segments of myelinated nerve fibers in living frog tadpoles have been observed in minute detail following many varieties of experimental irritation of both acute and chronic types. Illustrative ciné-photomicrographic records have been obtained. Below are specified the titles of some of the pictures:

1. Acute irritation and rapid metamorphosis of a myelin sheath segment immediately after a hot water burn. (A continuous picture showing myelin swelling, rippling, and ovoid formation.)

2. Swelling and wrinkling of the myelin sheath following administration of a strong anæsthetic (chloretone).
3. The production of beaded myelin by alcohol intoxication.
4. The swelling reaction shown by the same two myelin segments photographed immediately before and immediately after alcohol treatment.
5. Nerve irritation from low temperature (tadpole embedded in packed snow for several hours).
6. Examples showing trophic (Wallerian) degeneration of a myelinated fiber after section.
7. Irritation and recovery of a myelinated fiber following adjacent wound infliction.
8. Traumatic irritation and recovery of a proximal stump myelin segment after nerve section.
9. Examples of myelin segments showing delayed irritation, several days after exposure to X-rays.
10. Chronic irritation resulting from starvation. (Several examples of wrinkled and swollen nerves after six days total starvation.)
11. Granular degeneration of nerve fibers and sheath cells associated with prolonged inanition (20 days starvation).
12. Effects on nerves of administration of thyroid gland extract.
13. Pressure irritation showing delicate connecting strands between the axis cylinder and myelin sheath as these two structures undergo separation.
14. Temporary irritation and recovery of a myelin segment between two wounds, the nerve fiber remaining intact.
15. Polariscopic pictures showing the behavior of the anisotropic material of the myelin sheath during various stages of growth, irritation, and degeneration.
16. Degeneration of sheath cells on normal nerves.
17. Leucocytes, chiefly macrophages, moving about in a degenerating mixed nerve.
18. A macrophage leaving a blood capillary sprout (diapedesis).
19. A macrophage containing ingested anisotropic granules moving about within a single degenerating myelin segment. (Pictures taken with both ordinary light and polarized light.)

August 8, 1933

REGIONAL DIFFERENCES IN THE ORGANIZATION CENTER OF THE AMPHIBIAN EMBRYO. Edmund K. Hall. (Published in *Arch. f. entw. mech. d. organism.*, **127**: 573.)

OBSERVATIONS ON MIGRATING CELLS IN CULTURES OF AMPHIBIAN TISSUES, PARTICULARLY WITH REFERENCE TO THE PROBLEM OF FIBER FORMATION. George A. Baitsell.

THP EFFECT OF WING BUD EXTIRPATIONS IN CHICK EMBRYOS ON THE DEVELOPMENT OF THE NERVOUS SYSTEM. V. Hamburger.

COLOR CHANGES IN THE DOGFISH. G. H. Parker.

THE ABSORPTION OF COLLOIDAL CARBON BY THE MESONEPHRIC EPITHELIUM OF NECTURUS. A. B. Dawson.

MORPHOLOGICAL AND ELECTROPHORETIC EFFECTS OF THE GALVANIC CURRENT ON GRIFFITHSIA CELLS. Victor Schechter.—The morphological polarity of *Griffithsia* is established very early. After many nuclear divisions the cell divides once to form the shoot, and again to form the rhizoid. By continued rapid division branching chains of cells are formed. The basal cells are attached by means of rhizoids.

For the purpose of the experiments fragments of a thallus were placed in the experimental apparatus and oriented alternately with and against the current.

After two or three days of electrical treatment a very interesting picture could be seen.

When the fragment was oriented with base toward the anode, the rhizoids originated at the basal ends of the cells—that is, toward the anode, and the appearance of the completed thallus is more or less normal. However, if the fragment was oriented with the apex toward the anode, the rhizoids were again produced toward this pole and thus at the apical ends of the cells as shown. We have here a morphological indication of reversal of polarity.

Accompanying the gross morphological effect just described are correlated intracellular phenomena of unusual interest. First, there can usually be seen in the thallus as a whole a gradient of color ranging from a deep pink in the cells toward the cathode, more intense than the usual hue of the cells, to a pale tan in the cells toward the anode. A second intracellular effect is the aggregation of chromatophores and probably other cytoplasmic components toward the anode of each cell. It may be of significance in this connection that the first indication of rhizoid production under normal conditions is the formation of a visible pigment concentration, which then pushes out as the cap protoplasm of the rhizoid.

With regard to the color changes observed, it must be of some significance that rhizoid formation is inhibited in the region which, by virtue of its position, assumes the pale tan color. A pH change may be involved. This is supported by the fact that in acid the cell pigment approximates the deep pink color observed toward the cathode of the electric field, whereas in alkali it becomes pale and finally greenish in hue. Other evidence indicates that the electrical effect on color is not due to the production of acid or alkali in the cells but rather to a migration of materials which themselves may produce relative acidity or alkalinity by derived differences in concentration. With high currents each cell soon becomes deep pink toward the cathode and green toward the anode. If the current direction is now reversed, after a few minutes the colors reverse their position. Observations during the progress of this change show a fading of the pink at the anodal end *before* any pink color appears at the cathode. In this way there is a slow shift of pink intensity from anode toward cathode, pointing to the probability that the effect is not a pH change at the cell membrane.

The possible integration of these morphological and intracellular effects must be delayed pending further data. I wish only to point out at this time the coexistence of the described intracellular effects with the determinative action of the electric current during regeneration.

ELECTROKINETIC STUDIES OF MARINE OVA. I. ARBACIA PUNCTULATA. K. Dan. *Jour. Cell. Comp. Physiol.*, Vol. 3, No. 4. 1933.

ELECTRICAL ACTIVITY OF THE BRAIN. R. W. Gerard.

August 15, 1933

THE ISOLATION OF A CRYSTALLINE GLOBULIN FROM THE ALBUMIN FRACTION OF COW'S MILK. A. H. Palmer.

STUDIES IN IRON METABOLISM IN HUMANS. Paul Reznikoff.

THE HORMONAL CONNECTION BETWEEN THE PITUITARY AND THE THYROID. Marie Krogh.

ANTERIOR PITUITARY-LIKE HORMONE EFFECTS. F. E. Chidester.

August 22, 1933

HETEROCHROMATIC RADIATION AND EARLY AMPHIBIAN DEVELOPMENT. R. Rugh.—This report deals with radiation by light within the limits of the solar spectrum of the gametes and early developmental stages of the frog.

Distinction is made between luminous and energy intensities with the sugges-

tion that luminous flux is evaluated with reference to visual sensation and is limited to the total visible spectrum. It is not comparable to the objective physical entity of light energy measured as heat units. The two light factors controlled were light energy and wave length.

Frog gametes and fertilized eggs without jelly show the same limits of tolerance of direct sunlight. Since the pre-cleavage stages with jelly show much greater immunity to radiations than those without jelly, it is suggested that amphibian jelly may not concentrate the solar radiations but act as an insulator against them. Since jelly is 78 per cent water, it is to be expected that it would absorb heat rays.

An attempt was made to discover why monochromatic green has so often been reported as a lethal radiation, particularly in the work of Yung on tadpoles. A study of Yung's procedure indicated that neither monochromatic light nor equal energy radiations were involved. With these factors controlled, the quality of light within the limits of the visible spectrum did not matter so long as the total energy was equalized. It was suggested that green may have been lethal in Yung's work partly because he used plant food for the tadpoles and green plants cannot absorb green light, hence tadpole mortality was secondary to plant mortality.

A suggestion is made that radiation work distinguish between such factors as ultra-violet and total radiation from an ultra-violet lamp; light energy and the limited factor of luminous intensity.

A Non-linear Relation between Biological Effect and Ionizing Power of Alpha Rays. R. E. Zirkle.

Effects of X-rays upon Cell Oxidations. Leon C. Chesley.

A Response of Arbacia Eggs to X-rays. P. S. Henshaw and D. S. Francis. —*Arbacia punctulata* eggs when exposed to X-rays before fertilization are delayed in cleavage—that is, the interval between fertilization and the onset of the first cleavage is definitely prolonged. If the amount of radiation administered is varied, the amount of effect (cleavage delay) is found to vary in the same direction, indicating that the effect is a quantitative one. Further, if time is allowed to intervene between treatment and the measurement of effect (*i.e.*, between treatment and fertilization), the effect is found to be less than when measured at once after treatment; in other words, recovery in some form or other takes place. By plotting the amount of effect against the time allowed for recovery, a die-away type of curve is obtained. This shows that the rate of recovery is rapid at first but becomes less as time for recovery increases. When various degrees of effect are produced and recovery considered in each case, a family of recovery curves is obtained which seem to bear a relationship to each other. By placing the experimental points for such a set of data on a semi-logarithmic scale, parallel straight line curves are found to fit the points as well as any that can be drawn. This indicates that the rate of recovery is the same irrespective of the magnitude of effect produced and that the recovery process at least resembles a reaction which depends on the concentration of some substance—a mono-molecular reaction. Still further consideration shows that recovery is in progress even during treatment. From this it appears that X-rays cause a slowing in the rate of cell division in case of the first cleavage in *Arbacia* eggs and that recovery from the effect begins as soon as any effect is produced.

August 29, 1933

The Copepod Plankton of the Last Cruise of the Non-magnetic Ship "Carnegie." Charles B. Wilson.

The Pressure Coefficient of Viscosity in the Eggs of Arbacia. Dugald E. S. Brown.

OBSERVATIONS ON LACTIC ACID, TOTAL CO_2, AND pH OF VENOUS BLOOD DURING RECOVERY FROM SEVERE EXERCISE. Edwin P. Laug.

EYE COLORS IN THE PARASITIC WASP HABROBRACON AND THEIR BEHAVIOR IN MOSAICS AND IN MULTIPLE RECESSIVES. Anna R. Whiting.—Four independently segregating loci have been identified by recessive eye color mutations which are necessarily lighter than the black wild-type. The locus orange contains light-ocelli, dahlia, orange and ivory in order of decreasing pigmentation and dominance. White, in a second locus, is partially dominant to carrot. Cantaloup and maroon are independent.

In males (from binucleate eggs of heterozygous mothers) mosaic for eye colors in the orange series there is no sharp line separating the genetically different regions. The recessive region shows more or less of the dominant character at the margin, due apparently to diffusion of a chemical substance caused by the dominant allelomorph. In contrast, eye mosaics involving the white and cantaloup loci show a clearcut line between genetically different tissues and each region is obviously autonomous. No mosaics for maroon have been found. Of special interest is the eye, mosaic for factors in two loci, such as cantaloup and ivory. The cantaloup non-ivory region is clearly marked off from the non-cantaloup ivory region, while the latter shows black at the margin grading through orange to ivory. Thus the double dominant black, comparable to the eye color of the heterozygous mother, is physiologically reconstituted although there is no genetically black tissue present. If eyes are genetically ivory but other tissue (gonads by breeding test) is black, the eyes may be uniformly orange. Thus tissues of insects are not necessarily autonomous as has been frequently assumed.

Of the forty-eight combinations of eye color factors possible in haploid males, twenty-six have been made. Of these only nine are colored. It is probable that all other combinations would prove to be colorless. Intensity of color in a single mutant type appears to have little or no relation to its effect when in combination. Double recessives are lighter than the lighter single recessive.

THE MELANIN REACTION IN RACES OF DROSOPHILA MELANOGASTER. Marc A. Graubard.

August 31, 1933

HEMATOLOGICAL STUDIES IN DOGFISH (MUSTELUS CANIS). I. THE EFFECT OF TURPENTINE ON THE BLOOD PICTURE. Paul Reznikoff and Dorothy G. Reznikoff.—The average normal blood count of 25 dogfish per cu. mm. of blood was as follows:

Hemoglobin (Sahli) 33 per cent; range (21–44)
Red blood cells (Toissins) 393,000; range (210,000–630,000)
White blood cells (Toissins) 97,000; range (60,000–178,000)
Differential (supravital stain)
Small lymphocytes 54 per cent; range (35–71)
Large lymphocytes (granulocytes) 9 per cent; range (2–17)
Thrombocytes 18 per cent; range (6–32)
Eosinophiles 14 per cent; range (5–32)
Pseudoeosinophiles 4 per cent; range (1–8)
Monocytes 1 per cent; range (0–2)

Frequent removal of blood from the heart (0.5 to 0.75 cc.) may or may not cause an anemia and a reduction in white blood cells depending, probably, upon the amount taken. The differential count remains unchanged. Injection of sterile water intraperitoneally has no effect upon the blood picture.

Turpentine (0.25 to 1.5 cc.) injected intraperitoneally may or may not cause

some anemia but invariably causes a leukopenia with an increase of lymphocytes and a decrease of thrombocytes and eosinophiles. Just before death the thrombocytes may again rise. The following chart illustrates an example:

Time of blood examination	Hemoglobin	R.B.C.	W.B.C.	Small lymph	Large lymph	Thrombocytes	Eosin	Pseudoeosin	Monocytes
	per cent								
Before injection	39	359,000	127,000	37	9	20	32	1	1
1 cc. turpentine injected intraperitoneally									
12 hrs.	35	393,000	70,000	79	3	13	4	1	0
24 hrs.	25	308,000	71,000	71	9	12	5	3	0
36 hrs.	36	432,000	75,000	80	4	11	4	1	0
Fish dead 40 hours after injection									

An exudate of phagocytic lymphocytes and bacteria in the peritoneal cavity in the injected as well as the control fish is found after death.

These experiments indicate that the lymphocyte is the actively responding cell to acute irritation in the dogfish and has the same significance as the neutrophile (polymorphonuclear) in mammals.

INTRACRANIAL PIGMENTATION IN TELEOSTS. W. H. F. Addison.

THE BLOOD CAPILLARY IN RELATION TO CONTRACTILITY. E. R. Clark and Eleanor Linton Clark.—Federighi (1921), working under G. H. Parker, found in an invertebrate, *Nereis virens,* that the endothelial cells of certain blood-vessels possess a high degree of contractility; a peristaltic contraction which propels the contained blood.

Studies by the authors (1925) have shown that the endothelial cells of blood capillaries in the transparent tails of larvæ of several species of amphibians have a contractility which is decidedly less than that described for *Nereis,* but which is still a real activity which plays a part in controlling the circulation in the tail fins. No definite muscle cells are present on the arterioles or capillaries in this region, but extraendothelial cells are found which have been included by Vimtrup (1922) among cells which he calls "Rouget" cells, but which we have found noncontractile.

The morphology and behavior of blood capillaries in the rabbit's ear have been studied in our laboratory for the past seven years, by the use of double-walled transparent chambers, in which the arterioles, capillaries, and venules, including the cells making up their walls, may be seen with the greatest clearness with the highest microscopic lenses. Both the original capillaries of the ear and newly formed ones have been investigated, and it has been found by Sandison (1932) and by the authors in observations which have continued to date that the endothelial cells here are devoid of contractility, as are also the numerous "Rouget" cells. Local control of the circulation apparently resides exclusively in the arteries, arterioles, arterio-venous anastomoses and some of the larger veins, all of which are supplied with smooth muscle cells; their activity in turn depending upon an intact nerve supply.

These three sets of observations on invertebrate, amphibian, and mammal suggest an evolutionary loss of endothelial contractility associated with the elaboration of the neuro-muscular apparatus.

STRIATED MUSCLES OF THE LAMELLIBRANCH MOLLUSC, PECTEN GIBBUS. Herbert L. Eastlick.—A cytological study of the anterior portion of the adductor of *Pecten gibbus* shows that this muscle is composed of definitely striated myofibrillæ, the fibrillæ varying in size from barely discernible strands up to fibers 6 mμ in diameter. The segmented structure of the muscle is very apparent in both living and in fixed and stained preparations. A myofibril may be analyzed into the following components which seem to be homologous with similar structures in vertebrate or other highly differentiated muscle: the (Q) disc or the anisotropic segment, which is highly refractive and becomes intensely stained with hæmatoxylin; alternating with this disc is the (J) or the isotropic segment which is not very refractive and never stainable with hæmatoxylin. In the center of the (Q) disc there occurs a light area, the (H) disc or the disc of Hensen. The (J) disc is bisected by the (Z) line or membrane which is often granular and irregular in outline. In late stages of contraction and in complete contraction, never in the relaxed state, one can discern that the (H) disc is bisected by an exceedingly narrow line, the (M) membrane.

The striated portion of the adductor of *Pecten* differs from ordinary segmented muscle in that the fibrillæ are not organized into definite fibers, a true sarcolemma being absent and its place taken by a ramification of connective tissue which divides the muscle into irregularly sized bundles. Furthermore, the (Z) membrane is not continuous transversely across a muscle bundle but is limited to each myofibril; hence there is no definite transverse orientation of the discs and membranes as is always the case in vertebrate muscle.

Nuclei lie scattered at the inner surface of the connective tissue envelope surrounding a muscle bundle and in the interstices of the fibrillæ. The nuclei are spindle-shaped and in fixed preparations have an average size of 3×5 mμ. In the immediate area of a nucleus a clear, non-granular area of sarcoplasm occurs.

THE CENTRIOLES OF AMPHIBIAN TISSUES. Arthur W. Pollister.

THE CHROMIDIUM IN ARCELLA VULGARIS (EHRENBERG). Theodore G. Adams.—The investigation of the chromidium was made using the Feulgen reaction, Bauer's modification of the Feulgen reaction, and the mitochondrial technique of Benda.

In *Arcella* the Feulgen technique after the usual fixatives shows numerous granules of a deep purple color in the net. The network itself does not give the reaction. Methylene blue, Delafields' and Heidenhein's hematoxylin stain only the network leaving granules as clear discrete bodies. In a recent paper of Hans Bauer (1932), it is shown that chrome fixatives give better results with the Feulgen reaction that is a deeper color than when the aqueous solution of sublimate was used in Feulgen's original paper.

Bauer (1933), using the Schiff reagent, devised a test for polysaccharides. It consisted of chroming the tissue and applying the Schiff reagent without any hydrolysis at 60° C. He obtained reactions in the tissues of *Lumbricus* sp., *Helix pomatia*, and *Allium cepa* respectively for glycogen, galactogen, starch tunicin, and fructose. Inulin, a polysaccharide, did not give the reaction so that it is to be considered a presumptive test for a polysaccharide. In *Arcella* in the same clone the granules in the net gave a deep purple reaction to the foregoing test. No other structure in the cell gave the reaction. When the chroming process is supplemented by osmic acid without fat solvents in large quantities, fats and fatty substances such as Golgi material and mitochondria are preserved. These, according to Bauer (1933), react to the polysaccharide test when fixed in the forementioned manner.

In *Arcella* after the Benda fixation, bodies in the cytoplasm larger than the chromidial granules in the net react to the Bauer test for polysaccharides, with the exception that they are reddish, not purple. Some are paired unequal in size, some are equal, some are single. Some lie close to the net and have the appearance

of secondary nuclei, some adhere to the nucleus, a phenomenon characteristic of the surface tension effects of fats.

Animals from the same culture, previously examined for large granules in the cytoplasm, were fixed in a 4 per cent aqueous chromic acid and 20 per cent acetic and treated with Bauer's technique. The granules in the net gave a purple reaction but there was no trace of any bead-like or paired granules outside the net.

The living cells were stained vitally with Janus green B (Farbwerk Hoechst). It was difficult to see the characteristic color as differing from the original refractive index of the granules in the cytoplasm, outside the net.

The Benda technique stained these paired granules and other large granules in the cytoplasm outside the net a purplish red. This indicates the mitochondrial nature of the granules.

It may be concluded that the Bauer test is a presumptive test of a polysaccharide for the granules in the net of *Arcella* when fat solvents have removed fatty substances from the cell.

Selective Fertilization in Styela. Harold H. Plough.—Morgan found that removal of the chorionic membrane with the test cells from the egg of *Ciona* made self-fertilization possible. He therefore concluded that there was no incompatibility between eggs and sperm of the same animal. This conclusion might have been expected to hold for other tunicates showing self-sterility, but such is not the case.

In *Styela* (*Cynthia*) *partita* not all animals are self-sterile, but the common result of a series of reciprocal crosses is a small number of selfed eggs and a large number of crossed eggs showing fertilization and development. Since *Styela* eggs can be secured in large numbers only by allowing isolated animals to shed normally, all eggs are exposed to their own sperm before crosses are made. It turns out that with adequate controls the resultant exposure to a mixed sperm suspension in crosses is favorable for deciding the question of incompatibility of gametes.

During the past three summers Miss Evangeline Alderman and I have been studying this situation in *Styela*. It has been found that decrease or increase of the hydrogen ion concentration of the sea water, varying the salt content, introduction of *Styela* body fluid, and other changes in the normal optimum sea water only reduce fertility all along the line without any influence on self- or cross-fertility. Allowing the eggs to stand in sea water usually results in increased self-fertility and decreased cross fertility, until at the end of 3 or 4 hours approximate equality is reached.

Attempts to remove the chorionic membranes of *Styela* eggs by use of capillary pipettes have been uniformly unsuccessful. However, this result has been accomplished in about 20 per cent of cases by the use of crab digestive juice as suggested recently by Berrill (1932). There is always a general toxic effect when this fluid is used to digest off the membranes, but in the best cases many eggs develop. Strangely enough, removal or weakening of the membranes has no effect on the relation of self- and cross-fertility. The membranes appear to have nothing to do with the block to self-fertilization in *Styela*.

Finally, examination of many stained sections of eggs fixed about three hours after the final sperm exposure gives a perfectly clear picture, since the sperms are relatively large. The selfed eggs have many sperm inside the membranes and test cells and in close contact with the egg cortex. Fertilization has not taken place, as shown by the presence of the intact first maturation metaphase. In the crosses, however, the sperm do penetrate, and the eggs go into *cleavage*. Thus self-sterility in *Styela* seems to be a true case of selective fertilization.

Relation of Temperature and Cleavage in Frog's Eggs. Meyer Atlas.

Disorientations of Development in Crepidula, Caused by Cold. E. G. Conklin.

CHANGES IN THE ARBACIA EGG IMMEDIATELY FOLLOWING FERTILIZATION, AS DETERMINED BY CENTRIFUGAL FORCE. Ethel Browne Harvey.—*Arbacia* eggs centrifuged 25–90 seconds after fertilization become more elongate and break into halves and quarters more readily (most batches) than unfertilized eggs. With very high speed (11,000 × gravity), they can be pulled apart before the elevation of the fertilization membrane, and the membrane may form subsequently over the parts, but is thinner over the centripetal poles. If centrifuged 1½–5 minutes after fertilization, they break still more readily and into many small pieces. In some batches, the fertilized eggs break more readily at every stage after fertilization than the unfertilized. In other batches, they break more readily only till a certain stage which varies with the different batches. By observation of unfertilized and fertilized eggs at the same time while rotating in the double-head centrifuge microscope devised by E. N. Harvey, it can be definitely determined that the fertilized eggs at all stages stratify less readily than the unfertilized and are therefore more viscous. The difference is slight in the early stages and becomes more marked later on. The greater ease of breaking apart of the just fertilized egg must be connected with surface changes rather than with changes in viscosity.

RECOVERY FROM X-RAY EFFECTS IN ARBACIA EGGS BEFORE FERTILIZATION AND ITS EFFECT ON DEVELOPMENT. P. S. Henshaw and Dorothy S. Francis.—At a previous meeting we have described a response of *Arbacia punctulata* eggs to X-rays. The results presented showed that the period between fertilization and the onset of the first cleavage is definitely prolonged if the eggs are exposed before fertilization; and further, that recovery from this effect takes place if time is allowed to intervene between treatment and measurement of the effect (*i.e.* between the end of treatment and the moment of insemination). For this consideration, recovery was measured at once after the interval allowed for this process because cleavage time begins at the time of fertilization. More recently we have determined the degree of development attained 48 hours after fertilization, in an attempt to find whether the recovery which took place before fertilization was in evidence at a later period. The results obtained showed that it is and in much the same way as when measured by the effect on cleavage time. The organisms which had had the least time for recovery were retarded the most in development and those which had had the most time for recovery were most advanced. A curve showing the rate of recovery, as measured by effects on development, resembled the one which was obtained by measuring the effects on cleavage time. Because of this similarity, it appears that whatever X-ray effect is present at the time of fertilization is fixed by fertilization and that no further recovery takes place—that is, as far as this particular irradiation effect is concerned.

ON THE RELATIVE SENSITIVITY OF ARBACIA EGGS AND SPERM TO VARIOUS ACIDS AND BASES. Anna K. Keltch, Lucille Wade, and G. H. A. Clowes.—At last year's meeting it was reported that *Arbacia* sperm appeared to be relatively resistant to butyric acid and sensitive to ammonia, whilst *Arbacia* eggs were relatively resistant to ammonia and sensitive to butyric acid. These experiments have been extended during this season to include a series of mineral and organic acids and bases and a series of alcohols. Exposures were made in isotonic saline, using concentrations of 1,000, 4,000 and 16,000 eggs per cc., and .004, .016 and .064 cc. concentrated sperm per cc. for periods of 1¼, 2½, 5, and 10 minutes.

The extent of survival was determined by returning eggs and sperm to sea water and fertilizing previously exposed eggs with normal sperm and using previously exposed sperm for fertilization of normal eggs. The results previously reported with butyric acid and ammonia were confirmed. Hydrochloric acid was found to differ strikingly from butyric acid, being highly toxic for sperm as compared with eggs.

A rough index of relative toxicity, obtained by dividing the concentration

required to exert a given toxic effect on the sperm by that required to exert a given toxic effect on the eggs, puts hydrochloric, caustic soda, ammonia, and the ethyl amines in a range of 0.2 to 0.1 or less; that is, they are from five to ten times as toxic for sperm as for eggs. Organic acids of the type of citric, tartaric, and oxalic fall in a range of 0.3 to 0.5, as does formic acid, but the subsequent members of the fatty acid series, acetic, propionic and valeric, also benzoic acid, fall in the same category as butyric with an index of 2 or more. Apparently weak organic acids possessed of a great capacity for cell penetration are relatively less toxic for sperm than for eggs.

Preliminary tests conducted with ethyl, amyl, and octyl alcohols give them an index in the range of 0.3 to 0.5, thus apparently excluding surface tension as an important factor in the relative toxicity to sperm and eggs.

VARIATIONS IN THE SENSITIVITY OF EGGS FOLLOWING FERTILIZATION. G. H. A. Clowes, Anna K. Keltch, and Lucille Wade.—Using the procedure outlined in previous paper, sensitivity tests have been conducted on *Arbacia* eggs at five-minute intervals from fertilization to the third division. The curves have been compared with Heilbrunn's viscosity curves and Fry's description of egg development. As reported at last year's meeting, butyric acid and ammonia, which penetrate the egg easily, exert effects after fertilization to the point of fusion of egg and sperm nucleus differing strikingly from the effects which they exert on the unfertilized egg but corresponding approximately with that exerted on sperm, the egg during this period being relatively sensitive to ammonia and resistant to butyric acid.

As development proceeds eggs become relatively less sensitive to ammonia and more sensitive to butyric. There are points of relative maximum sensitivity to ammonia which appear to fall in the lower zone of the viscosity curves and well-defined points of maximum sensitivity to butyric acid at points of maximum viscosity, viz., that following fusion of egg and sperm nucleus and those at which 50 per cent of the eggs have divided.

Oxalic acid differs strikingly from butyric acid, showing points of maximum sensitivity in the lower zone of the viscosity curves following the breakdown of the nuclear membrane, and resistance at the peaks of viscosity, suggesting the possibility that the calcium ion may play a rôle in connection with coagulative changes associated with spindle formation and cell division.

Hypotonic NaCl, like butyric acid, exerts a maximum destructive effect at high viscosity points. The addition of calcium may eliminate the hypotonic effect at the high viscosity point following fusion of egg and sperm nucleus, and may reduce but not eliminate the point of maximum sensitivity when 50 per cent eggs have divided.

The strikingly cyclic rhythms obtained with butyric acid and the somewhat less striking but divergent rhythms obtained with ammonia, which harmonize roughly with Heilbrunn's viscosity curves and also with the periods of low intensity of staining of chromatin in the intact nucleus and high intensity of staining of the chromosomes during division, suggest that some type of acid basic cyclic rhythm plays a significant rôle in the mechanism of cell division.

THE FLATTENING OF MARINE EGGS UNDER THE INFLUENCE OF GRAVITY. E. Newton Harvey.—If egg cells are considered drops of a non-miscible liquid in water, resting on a plane surface, they should flatten, provided the tension (T) at their surface is sufficiently low or the force [gravity $\times$ difference in density between egg and medium, g $(d\text{-}d')$] is sufficiently great, or their radius (r) is sufficiently large. Plasticity or elastic structure would act against flattening. Within limits the flattening, f, varies as $r \times \sqrt{g\,(d\text{-}d')}/\sqrt{T}$.

Previous experiments indicate that the tension at the surface of the cells is low—less than one dyne per cm. From Heydweiller's tables and equation, it can be calculated that an egg whose tension at the surface is 0.2 dyne/cm. should not

flatten 10 per cent unless its radius is over 400 μ. Since most marine eggs are smaller than this, we should not expect them to flatten appreciably unless T is very low. Observation from the side shows that "naked" eggs like *Arbacia, Asterias, Echinarachnius, Ilyanassa, Crepidula plana, fornicata* or *convexa*, and *Chætopterus* are either perfectly spherical, or, if irregular, remain so, without flattening. *Busycon* eggs (1 mm. diameter) are of the consistency of putty and do flatten sufficiently to indicate a tension at the surface of less than 0.5 dyne/cm. Eggs with a definite chorion at the surface may be spherical (*Phascolosoma*), slightly flattened when shed (*Nereis*), or disc-shaped when shed (*Arenicola*). Such eggs merely fall on their flat side and the flattening has nothing to do with the action of gravity.

THE ACTION OF ANÆSTHETICS ON THE SURFACE PRECIPITATION REACTION. L. V. Heilbrunn.

THE EFFECT OF CERTAIN SALT SOLUTIONS ON THE PERMEABILITY OF THE ARBACIA EGG. Dorothy R. Stewart and M. H. Jacobs.—It is generally believed that the absence of bivalent cations such as Ca^{++} from the medium surrounding a cell may cause an increased permeability of the latter. Accurate quantitative studies of this effect have been made in the case of water by Lucké and McCutcheon, but are almost lacking in the case of solutes. A method developed by one of the authors permits the simultaneous determination of "permeability constants" for water and for a penetrating solute. This method has been applied to the egg of *Arbacia*, using ethylene glycol as the penetrating solute, and comparing permeability in sea water, in isotonic solutions of NaCl, KCl, and $CaCl_2$, and in solutions containing various combinations of NaCl and $CaCl_2$. The results on the permeability of the unfertilized egg to water are in satisfactory agreement with those which Lucké and McCutcheon obtained by an entirely different method. They indicate a considerably greater permeability in NaCl and in KCl than in sea water and in $CaCl_2$. Unlike the permeability of the cell to water, that to ethylene glycol is almost the same in sea water and in the various salt solutions. The addition of $CaCl_2$ in increasing amounts to NaCl causes a progressive decrease in permeability to water while leaving that to ethylene glycol practically unaltered. In striking contrast with the effects of changing the electrolytes in the external medium are those of fertilization, which causes a great increase in permeability not only to water but to ethylene glycol as well. The fact that one type of treatment of the *Arbacia* egg increases its permeability to both substances and another type of treatment increases its permeability to water alone is of interest in connection with certain theories as to the nature of cell permeability.

THE EFFECTIVE PERIOD IN DEVELOPMENT OF THE MUTANT FACTOR "EYELESS" IN HABROBRACON. B. R. Speicher.—Eyeless wasps show malformed head capsules characterized by production of a pair of large lobes on the sides of the head which bear minute compound eyes at their tips.

In wild-type wasps the dorsal part of the head capsule is formed by four imaginal buds, an anterior pair, the antenna-cephalic discs, forming the antennæ and some hypodermis at their bases, and a posterior pair, the oculo-cephalic discs, forming the head capsule around and including the compound eyes and ocelli. These imaginal buds develop by invagination and separation from the larval hypodermis and proliferate within the larval head.

In eyeless, the antenna-cephalic discs and the anterior and ventral margins of the oculo-cephalic discs develop normally. During the late larval stage the dorsal and posterior margins of the oculo-cephalic discs fail to separate from the larval hypodermis and, being thus restricted in development, grow outward to form the lobes characteristic of the mutant type. Later development is normal except for some modification by previous abnormality. The compound eyes are not close to the optic lobes, and there are no nerve connections between the two organs.

VARIEGATED EYE COLOR IN HABROBRACON. Anna R. Whiting.—When the semi-dominant factor shot-veins is combined with the factor for white eye color (whwh.svsv), all the white eyes show red spots in the posterior ventral region. If the white-eyed animal is heterozygous for shot-veins (whwh.Svsv), the spots are reduced in extent. The variegated effect is therefore semi-dominant in respect to non-variegated. Variegated stock is fully fertile and stable. White and shot-veins have been combined with other mutant eye factors. Variegation shows in no combinations except with three allelomorphs in the orange series; type (O), dahlia (o^d), and orange (o) and with the carrot allelomorph to white. Wh.sv.O is typical variegated, wh.sv.o^d is light variegated, and wh.sv.o is very slightly variegated. White in combination with its allelomorph carrot and with shot-veins ($whwh^c$.svsv) produces typical variegation pattern on a cream background; with heterozygous shot-veins ($whwh^c$.Svsv) it gives slight variegation on cream background.

The dominance of shot-veins (and of its secondary effect, variegation) suggests that it is due to translocation but allows the possibility of its being a gene mutation. If due to translocation it must be stable since variegated is fully fertile even in haploid males and breeds true. Two suggestions are offered to explain the red spots. First: shot-veins factor or condition is associated with the constant habit of somatic mutation occurring at a definite stage in the development of the compound eye, such that only facets in the posterior ventral region are affected. Every eye is a mosaic (and a mosaic of rather constant pattern) according to this theory. Second: the shot-veins factor or condition has a spotting effect on the eyes when the residual heredity is such as to allow its expression and the cells of the red region are of the same genetic constitution as those of the white. The second theory is preferred by the author.

EGG-TRINUCLEARITY IN HABROBRACON. P. W. Whiting.—It has hitherto been assumed that mosaic males in *Habrobracon* develop from binucleate eggs in which the two nuclei correspond to the second polar nucleus and the reduced egg, the genetic difference between them resulting from post-reduction of those factors for which the mother was heterozygous. Several cases have now been obtained indicating egg-trinuclearity. Thus three of the four possible combinations of two pairs of allelomorphs have been shown in various parts of the body of the mosaic. At least three oötids from one oöcyte may therefore take part in parthenogenetic cleavage. Breeding tests have thus far shown not more than two of the possible combinations to be present in the sperm produced by the mosaic.

RELATION BETWEEN OXYGEN TENSION AND RESPIRATION IN SPIROSTOMUM AMBIGUUM, WITH CORRECTIONS FOR AMMONIA. Heinz Specht.—The rate of consumption of oxygen and of production of carbon dioxide in the ciliate, *Spirostomum ambiguum* (Saars), was measured under normal, low, and high tensions of oxygen by means of the Barcroft manometer (Gerard's modification, *A. J. P.*, 1931) following the technique of Warburg (*Bioch. Z.*, 1923 et seq.) with HCl in an additional chamber in the manometer vessel for the purpose of absorbing ammonia. Evidence is presented showing that ammonia is evolved, and that the correction for its evolution is essential in measuring the respiration of any tissue that produces it in appreciable quantities.

In air the average rate of consumption of oxygen and production of carbon dioxide per hour per thousand animals is respectively 2.59 $mm.^3 \pm 0.41$ $mm.^3$ and 2.17 $mm.^3 \pm 0.66$ $mm.^3$ when the evolved ammonia is absorbed by HCl. Without the acid the values are respectively 2.10 $mm.^3 \pm 0.9$ $mm.^3$ and 0.86 $mm.^3 \pm 1.23$ $mm.^3$. In an atmosphere of nitrogen containing 0.5 per cent oxygen the average rate of consumption of oxygen and production of carbon dioxide per hour per thousand animals is respectively 1.86 $mm.^3 \pm 0.95$ $mm.^3$ and 2.53 $mm.^3 \pm 0.81$ $mm.^3$ until disintegration of the animals sets in, then the values are respectively 0.07 $mm.^3 \pm 0.44$ $mm.^3$ and 2.79 $mm.^3 \pm 2.04$ $mm.^3$. Without HCl the values under these

conditions and over the same periods are respectively 0.76 mm.^{3} $\pm$ 0.28 mm.^{3}, 0.76 mm.^{3} $\pm$ 0.89 mm.^{3}, and 0.74 mm.^{3} $\pm$ 0.41 mm.^{3}, 3.24 mm.^{3} $\pm$ 1.06 mm.^{3}. This slight rise in the rate of production of carbon dioxide after disintegration sets in indicates a process which is not necessarily life-supporting, *i.e.*, not anoxybiotic but simple anoxic. In a concentration of oxygen of about 100 per cent the average rate of consumption of oxygen and production of carbon dioxide per hour per thousand animals is 3.92 mm.^{3} $\pm$ 0.79 mm.^{3} and 3.80 mm.^{3} $\pm$ 0.14 mm.^{3} respectively. This high rate is accompanied by a progressive injury to the animals and rises but slightly when they disintegrate. *Spirostoma* subjected to low tensions of oxygen for two hours and then furnished with oxygen at the normal tension exhibit a rise in the rate of respiration which approaches that in pure oxygen.

Increasing the oxygen tension 5 times over the normal value in air causes an increase to 1.5–1.7 times normal respiration, while decreasing it 40 times under the normal tension causes a decrease in consumption of oxygen to 0.7 times the normal value and an increase in production of carbon dioxide to 1.17–1.29 times the normal value.

It is concluded that the respiration of *Spirostomum* is nearly independent of oxygen tension down to very low values.

Luminescence and Respiration of Bacteria in Carbon Monoxide. C. S. Shoup.—Carbon monoxide inhibits the respiration of luminous bacteria, but produces no significant loss of luminescence down to a decreased respiratory rate of only 45 per cent of an identical control suspension in $N_2 + O_2$ instead of $CO + O_2$. Luminescence and respiration are independent of each other in the presence of CO and low O_2-pressures down to the point of dimming due to oxygen-lack alone (0.26 per cent oxygen: Shoup, 1929). There is some evidence of a partial recovery from CO-narcosis.

The Relation between Luminescence and Respiration in Bacteria with Especial Reference to the Effects of Narcotics. G. Wellford Taylor.—If bacterial luminescence were dependent upon respiration, variations in the respiratory rate should be accompanied by similar variations in luminescence. This was not found to be the case in studying the effects of narcotics and certain other substances. In this work marine luminous bacteria were used as their own indices of rate of oxygen consumption by means of the dimming method of Harvey. Luminous intensities were measured on a photometer bench.

It was found that low concentrations of narcotic stimulated luminescence, higher concentrations produced reversible narcosis, while still higher concentrations caused irreversible changes. The same relation of effect to concentration holds for respiration but the concentrations are much higher than for luminescence, so that concentrations sufficient to markedly inhibit luminescence may stimulate respiration. In this way luminescence has been decreased 50 per cent while at the same time respiration increased 100 per cent.

A further separation of the two processes has been effected with KCN, with which respiration has been reduced 60 per cent without decreasing luminescence.

It has also been observed that when a fresh suspension of bacteria is allowed to stand, with aeration, its luminescence and respiration vary with time, but they do not vary together. The luminescence may increase 100 per cent without any increase in respiration; the respiration may decrease 45 per cent without affecting the luminescence.

The amount of nutrient material in a suspension is an important factor in determining the narcotic effect of respiration. By controlling this factor the stimulation of respiration by a given concentration of narcotic has been varied from 3 to 100 per cent, the stimulation increasing as the amount of nutrient decreased. This suggests that in addition to the stimulation of respiration per se, the narcotic may be used as food material by the bacteria. There is other evidence to support this view.

From these observations it appears that within wide limits luminescence is independent of respiration.

NATURE OF THE AEROBIC APPARENT REDUCTION POTENTIAL. Lyle V. Beck.

THE RELATIVE ABUNDANCE OF HYDROGEN ISOTOPES IN SEA WATER. Eric G. Ball.—The ratio $H^2 : H^1$ in Berkeley city water has been calculated by Lewis and Macdonald to be of the order 1 : 6500. Bleakney and Gould have given the ratio in rain water as 1 : 5000. The apparent inability of living cells to utilize water composed of the heavier hydrogen isotope (Lewis) demands the investigation of water from other natural sources for variations in this ratio. It seemed possible that an increase in the concentration of the heavier hydrogen isotope might occur with increasing depths in the ocean.

Two samples of sea water were employed. One sample, kindly supplied by Dr. A. C. Redfield, was collected in the Caribbean Sea at a depth of 4500–5000 meters during a recent voyage of the "Atlantis." The other sample was Woods Hole surface water. Distilled water was used for comparison. All samples were distilled five times from an all Pyrex still, freed from dissolved air, and the specific gravity determined in a 100 ml. pycnometer. The specific gravity of all three samples agreed within the experimental error of $\pm 5 \times 10^{-6}$. It therefore appears that the relative abundance of the hydrogen isotopes in sea water from the surface or from great depths is of the same order of magnitude as that previously reported for fresh water samples.

TOXICITY OF SOME METALS AND BERKEFELD FILTERED SEA WATER TO MYTILUS EDULIS. Oscar W. Richards.—Three young (5–8 mm.) mussels were placed in a finger bowl with 100 ml. of sea water and the metal to be tested. The water was changed about every three days during the 41 days of the experiment and the temperature averaged 22° C. When an animal died it was replaced. In the following table of data the growth of the animals is expressed as a percentage of that of the control animals and the toxicity of the metals is given as the average time required to kill all three of the animals.

Source	Material	Type	Form	Growth	No. dead	Remarks
				per cent		
Control	—	—	—	100	1	—
Newark Wire Cloth Co.	Monel	—	screen	none	14	Kills in 1–2 days
Newark Wire Cloth Co.	Aluminum	pure	screen	100	none	No corrosion
Newark Wire Cloth Co.	Stainless steel	KA2-S	screen	106	1	No corrosion
Newark Wire Cloth Co.	Phosphor bronze	—	screen	none	9*	Kills in 20 hours. Highly corroded
International Nickel Co.	Inconel	(80% Ni, 14% Cr., 6% Fe)	strip	24	3	Slightly corroded
International Nickel Co.	Nickel	Finish No. 8	strip	30	4	No corrosion
International Nickel Co.	Monel	—	strip	none	11	Kills in 1–3 days
J. Bishop & Co.	Tantalum	—	spinnets	60	3	No corrosion
U.S.B.F.	Nickel	—	wire	83	1	No corrosion
Precision Sci. Co.	Brass	—	clamp holder	none	14	Kills in 2–3 days. Moderate corrosion
Fisher Sci. Co.	Castaloy	—	clamp holder	none	12	Kills in 3–4 days. Highly corroded
Berkefeld candle immersed in sea water	—	—	—	none	14	Kills in 2–3 days
50 ml. Berkefeld filtered sea H_2O + 50 ml. sea H_2O	—	—	—	18	2	—
75 ml. Berkefeld filtered sea H_2O + 25 ml. sea H_2O	—	—	—	1	4	—

* Discontinued after 19 days.

Berkefeld filtered sea water is not satisfactory for the dilution of available food in sea water for *Mytilus* in growth studies. Aluminium is non-toxic but not strong enough for the construction of live boxes. Stainless steel and nickel, although somewhat more expensive than the usual asphalt-covered galvanized iron, are non-toxic and could be used for the screen covering of live boxes. However, the former will rust slightly at the water line when it is not completely immersed in sea water.

SOME NEW ASPECTS OF THE NERVE-MUSCLE PHYSIOLOGY IN CRUSTACEA AS SHOWN BY TIME AND INTENSITY FACTORS IN ELECTRICAL EXCITATION AND RESPONSE. Herbert H. Jasper.*—Chronaxie values of 0.6 to 1.0 σ are obtained for the appearance of the first action potential in isolated claw nerves (crabs and lobsters). The apparent chronaxie, for a threshold (visible) contraction of the adductor claw muscle, ranges from 8 to 20 σ.

Nerve action potentials (with cathode ray oscillograph) show a rhythmic response to single constant current stimuli slightly above the intensity and time threshold for the appearance of a single wave. The frequency of nerve response (within certain intensity and time limits) is very regular for the complete duration of the current flow and increases with intensity up to the limit imposed by the refractory period. Threshold muscle contractions are observed only with stimuli which elicit a certain number and frequency of nerve response which explains the large discrepancies observed between nerve and muscle chronaxie measurements. The apparent "chronaxie" of isolated nerve muscle approaches a "temps de sommation" and cannot be called a chronaxie.

The experiments of Keith Lucas, which tended to show a slow and fast nerve-muscle system in *Crustacea*, have been repeated with most of the experimental facts being verified although extended in the light of the rhythmic response of the nerve to single stimuli and the consequent tetanic nature of muscle response which explain all the phenomena observed without the necessity of the hypothesis of a dual nerve-muscle mechanism. The slow and fast nerve action potentials found by Monnier and Dubuisson are probably due to the potentials from sensory and motor nerves and not to a double motor system since we have found that the slow wave is elicited upon sensory stimulation.

It was found that adductor and abductor claw muscles as well as nipper and crusher muscles in the lobster differed in their speed of contraction with corresponding differences in summation time and frequency for fused tetanos. Since these differences in rapidity of excitation and response seem to be associated with histological differences in myomere size, we draw the conclusion that excitation and inhibition in crustacean muscle as well as speed of response is a function of the intensity and time characteristics of the end organs; the latter being qualitatively not unlike all other striated muscle if the experiments of Bremer on summation in frog muscle are taken into account.

THE BLOOD PRESSURE OF LIMULUS. Margaret Sumwalt and Kathryn McLane.—In the frontal artery of *Limulus*, close to its origin from the heart, the most frequently observed mean blood pressure is 16 cm. H_2O. The range of all the variations observed among different individuals, or in the same individual with bouts of struggling, is included between 6 and 32 cm. H_2O. The pulse rate is between 15 and 39 beats per minute, and appears to be correlated inversely with the mean pressure.

The figures are based on study of 19 full grown unanæsthetized males. An animal fresh from running sea water is tacked through the margin of the carapace to a board. Removal of a square inch of the shell (usually without hemorrhage) exposes the artery. A cannula filled with mineral oil connects the artery with a mercury manometer, the meniscus of which is photographed, and with a straight

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erect oil manometer for calibration. (The ordinary anticoagulants are ineffective against the type of clotting which occurs in *Limulus* blood, whereas oil in the cannula does somewhat delay it.) The oil manometer is secondarily calibrated against a water manometer. Some of the data summarized above were obtained by direct observation of the oil manometer against a scale, some by analysis of photographic records. The error by either method does not exceed 2 cm. H_2O.

The observations were made as a contribution toward a knowledge of the comparative physiology of water balance among the invertebrates. If the Starling hypothesis, which governs present ideas of water balance in the vertebrates, is also applicable to such of the invertebrates as possess a closed circulatory system, a direct relation should be found between the colloid osmotic pressure of their bloods and the systemic blood pressure. Very few measurements of either sort of pressure in an invertebrate are now on record.

THE EFFECT OF pH UPON POTASSIUM PENETRATION INTO FUNDULUS EGGS. John C. Bridges and Margaret Sumwalt.—Using standstill of the embryonic heart as a criterion for the presence of a definite concentration of K in the egg, we have found that increasing acidity, within the pH range down to about 3.6 where acid injury begins to occur, decreases the penetration of this cation. The time required to stop 50 per cent of the hearts with M/2 KCl is 60 minutes at pH 4, 38 minutes at pH 5, 36 minutes at pH 6, and 34 minutes at pH 7. Unbuffered solutions were used.

The results are interpreted to mean that acidity decreases the permeability of the egg to K. At least part of this increased permeability is believed to occur at the chorion. The relative rates of penetration of various ions through the chorion of the *Fundulus* egg have already been determined by the method of measuring potential differences. It appears that the chorion in neutral solutions is more permeable for cations than for anions, but that with increasing acidity its ion favoritism is gradually withdrawn and finally reversed. It would be interesting to know whether the withdrawal of membrane influence by acid permits an increased penetration of anions, or reduces the movement of cations, or does both to different extents; but the permeability of a membrane for ions in any absolute sense cannot be gauged by measurements of potential differences. For this particular cation, K, the present experiments indicate that acidity decreases the absolute penetration.

A METHOD FOR FOLLOWING VOLUME CHANGES OF CELLS. Arthur K. Parpart.

THE INFLUENCE OF pH UPON THE PASSAGE OF HEMOGLOBIN THROUGH THE GLOMERULUS OF THE PERFUSED FROG'S KIDNEY. William R. Amberson, Frank Engel, Dorothy Webster, and Edwin P. Laug.—Hemoglobin in solution in the blood stream of vertebrates leaves fairly rapidly through several exits. Most of it is lost through the kidney, and its disappearance leads to the death of animals whose normal blood has been replaced by hemoglobin-Ringer, a solution containing a quantity of hemoglobin from hemolyzed beef blood cells.

In an attempt to discover a method which may minimize or prevent the loss of hemoglobin into the urine, we have begun a study of various physico-chemical factors involved in the glomerular elimination of hemoglobin, and are now able to report on the influence of the H-ion concentration. We have perfused the kidneys of large bull frogs through aortic cannulæ, and have been able to secure readings relating the hemoglobin in the urine to that in the perfusion fluid over a pH range from 5.2 to 7.9. We find that hemoglobin passage is very much greater on the acid side of the isoelectric point of hemoglobin than on the alkaline side. The hemoglobin in the urine is about 10 per cent of that in the perfusion fluid, on the average, at pH 7.2 to 7.9, whereas it reaches about 30 per cent between pH 6.0 and 6.6, and rises to as high as 70 per cent at more acid values. The effect is reversible. Thus a high hemoglobin may be obtained with a perfusion at a low pH, to be suc-

ceeded by a low amount at a more alkaline value, in the same kidney. We have been able to reverse the effect up to five times.

We are inclined to explain the effect as being related to the charge of the hemoglobin ion, although a reversible change in the glomerular membranes is not ruled out. It seems possible to us that, on the alkaline side of its isoelectric point, the negatively charged hemoglobin ion may find it relatively difficult to pass through negatively charged glomerular membranes, but may penetrate with ease when its charge is reversed. If this is correct it suggests that other factors than the large size of the protein ions are involved in their penetration through living membranes.

THE INFLUENCE OF THE ESCAPE OF SALTS ON THE OSMOTIC BEHAVIOR OF THE ERYTHROCYTE. M. H. Jacobs and Arthur K. Parpart.—Certain deviations of the behavior of the erythrocyte from that of a perfect osmometer have been explained by Ponder and his collaborators as being due to an escape of salts from the cell. While such an escape is probable in experiments of sufficiently long derivation, it is shown that the osmotic properties of the erythrocytes of the ox, as studied by the hemolysis method, do not seem to undergo an appreciable change in times shorter than several hours. More specifically, the degree of hemolysis produced by a given hypotonic NaCl solution is not decreased by a preliminary exposure of from a few seconds to two or three hours to a slightly less hypotonic solution, in itself just unable to cause hemolysis. An increased osmotic resistance, suggestive of the escape of salts, is, however, produced by longer exposures. The properties of the erythrocytes of the pig, the cat, the rabbit, and man change more rapidly and these cells are therefore less favorable for certain types of osmotic studies than those of the ox.

In solutions of sucrose, either hypotonic or isotonic, there is a very rapid increase in the osmotic resistance of erythrocyte. This increase occurs in two stages: a very rapid one and a much slower one which follows the first after a considerable interval. While the slow change may well be associated with an escape of salts, the more rapid and more striking initial one is interpreted as the effect of an ionic exchange. In favor of this interpretation is not only the rapidity of the process but also its reversibility. The ionic exchange in question is believed to be one of anions from the cell for OH' ions from the solution, the resulting increase in base bound by hemoglobin causing a decreased osmotic pressure within the cell.